RESULTS

RESULTS

Total Blood Protein :

Applying three doses of iron (as ferric chloride) manganese (as manganous chloride), zinc (as zinc sulphate) and copper (as copper sulphate) to fish groups resulted in an increase of the mean total serum protein of oreochromis niloticus (Table 1) .However, the high iron dose (515.4 mg/l) was lehtal to the fish where all of them died within few hours. The other two doses (low and intermediate) produced a highly significant ($p<0.001$) increase of total serum protein at the end of the test period as compared with that of the control fish group. The increase was 131.4% and 222.8% of the control group for the low and intermediate iron dose treated fish, respectively.

Manganese treatment also caused an increase of the mean total serum protein of O. niloticus as compared with that of the cotrol fish group. The mean total serum protein was 6.4±0.2, 7.1±0.2 and 7.5±0.1g/ 100 ml for the low, intermediate and high manganese doses respectively compared to 3.5±0.1g/100 ml for the control group. This increase was statistically highly significant ($p<0.001$)and the percentage of increase reached 82.8%, 102.8% and 114%, respectively (Table 1).

Total serum protein of the Nile fish; Oreochromis niloticus increased also after raising the fish for one week period in any of the doses of zinc (as zinc sulphate). The mean total serum protein value of the control fish was 3.5±0.1g/100 ml which was increased to 5.56±0.4,

8.40±0.3 and 10.3±0.5 g/ 100 ml for the low, intermediate and high zinc doses, respectively. It is clear that the total serum protein of O. niloticus treated with the three doses of zinc was highly significantly increased ($p < 0.001$) and this increase reached 58.8%, 140% and 194%, respectively.

Similarly the mean value of the total serum protein of O. niloticus exposed to any of three levels of copper (as copper sulphate) was increased significantly ($p<0.001$) as compared with that of the control fish group. This increase was 61% for fish group treated with low copper dose, 80% for fish group treated with intermediate copper dose and 160% for fish group treated with high copper dose, respectively.

It is obvious from table (1) that applying the four metals (iron-manganese, zinc and copper) at different doses to fish groups resulted in an increase in the mean value of total serum protein of the Nile fish; O. niloticus,

Table (1): Mean and range values of total serum protein (g/100ml) of the blood of Oreochromis niloticus after exposure to three concentration levels of each of iron, manganese, zinc and copper (as salts) for 7 days (7 animals were used per test).

	Control	Iron	Manganese	Zinc	Copper
Low dose					
Mean ± S.E.	3.50 ± 0.10	8.10 ± 0.30	6.40 ± 0.20	5.56 ± 0.40	5.6 5± 0.10
Range	3.10 — 4.0	7.0 — 9.0	5.40 — 7.00	4.0 — 7.0	5.0 — 6.10
Significance		P<0.001	P<0.001	P<0.02	P<0.001
% of change		+ 131.4%	+82.8%	+58.8%	+61%
Intermediate dose					
Mean ± S.E.		11.30±0.40	7.10±0.20	8.40±0.30	6.3 ± 0.20
Range		10.0—12.0	6.20—7.80	7.10—10.00	5.20 — 7.00
Significance		P<0.001	P<0.01	P<0.001	P<0.001
% of change		+222.8%	+102.8%	+140%	+80%
High dose					
Mean ± S.E.			7.50 ± 0.10	10.30±0.50	9.10±0.30
Range		All fish expired	7.0 — 8.0	8.20—12.50	8.50—10.20
Significance			P<0.01	P<0.001	P<0.001
% of change			+ 114%	+194%	+160%

Blood Serum Enzymes :

Effect of Iron :

The activity of each of glutamate oxaloacetate transaminase (GOT) , glutamate pyruvate transaminase (GPT) (IU/min/l) and acid (AP) and alkaline (AlP) phosphatases (kind and king Units/l) enzymes of the serum of Oreochromis niloticus treated with three doses of iron as ferric chloride are shown in table (2).

It is evident that the mean level of GOT of the control O. niloticus was 27.0±0.7 IU/min/l where as that for fishes raised in the low iron dose for one week was 39.0±0.4 IU/min/l while that of those kept in the intermediate iron dose was 53.6±0.7 IU/min/l. Fish exposed to the high dose of iron (515.4 mg/l) were expired after few hours of subjection to that metal salt concentration. Statistically the increase of the mean level of GOT activity was highly significant ($p<0.001$) for both of the low and intermediate doses as compared with that of the control group. This increase amounted to 44% and 98% in case of fishes treated with the low and intermediate doses respectively.

The mean level of GPT activity of the serum of O. niloticus of the control fish and fishes exposed to the low and intermediate doses of iron was 9.0±0.4, 16.5±0.3 and 20.8±0.7 IU/min/l, respectively. Statistical analysis revealed that the increase in the mean level of GPT activity was highly significant ($p<0.001$) for the low and intermediate doses respectively. The percentage of increase was 83% for the low dose exposed fish and 131% in the case of intermediate dose exposed ones (Table 2).

It can be seen from the table that there was an increase in the mean level of acid phosphatase (AP) activity that reached 5.7±0.05and 7.1± 0.08 kind and king Units/l compared with 2.8±0.07 kind and king Units/l for the control group fishes. Statistically this increase of AP mean activity was highly significant ($p<0.001$) and the percentage of increase reached 103% and 153% in the case of fish exposed to the low and intermediate doses respectively.

Furthermore, serum alkaline phosphatase (AlP) mean level of activity of the control fish group was 21.0±0.4 kind and king Units/l while that for fishes raised in the low dose of iron was 53.3±0.05 kind and king Units/l and that of fish exposed to the intermediate dose was 59.6±0.5 kind and king Units/l. It is evident that the increase of the mean AlP activity was highly significant ($p<0.001$) in both treated groups. This increase was 153% and 183% in case of fish exposed to low and intermediate doses of iron respectively.

It is evident from the previous data that there was an increase of the mean level of activity of all of above mentioned enzyme activities of the serum of O. niloticus subjected to the two doses of iron (as ferric chloride) and this increase was more deeper with the increase of the dose of iron. However, fish subjected to the third dose (high dose) died within few hours of treatment.

Table (2): Mean and range values of serum glutamate oxaloacetate transaminase in (GOT), glutamate pyruvate transaminase (GPT) (IU/min/l) and acid (AP) and alkaline (Alp) phosphatases (kind and king Units/l) activities of Oreochromis niloticus after exposure to three concentrations of iron (as ferric chloride) for 7 days (7 animals were used per test).

	GOT	GPT	AP	AlP
Control				
Mean ± S.E.	27.0 ± 0.70	9.0 ± 0.40	2.80 ± 0.07	21.00 ± 0.40
Range	24.0 — 29.0	7.0 — 10.0	2.50 — 3.0	20.0 — 22.0
Low dose				
Mean ± S.E.	39.0 ± 0.40	16.50 ± 0.30	5.70 ± 0.05	53.30 ± 0.50
Range	39.0 — 41.0	95.0 — 17.0	5.50 — 5.90	51.0 — 55.0
Significance	P < 0.001	P < 0.001	P < 0.001	P < 0.001
% of change	+ 44 %	+ 83 %	+ 103 %	+ 153 %
Intermediate dose				
Mean ± S.E.	53.60 ± 0.70	20.80 ± 0.70	7.10 ± 0.08	59.60 ± 0.50
Range	51.0 — 55.0	17.0 — 22.0	6.90 — 7.50	58.0 — 62.0
Significance	P < 0.001	P < 0.001	P < 0.001	P < 0.001
% of change	+ 98 %	+ 131 %	+ 153 %	+ 183 %
High dose				
Mean ± S.E.				
Range	All fish expired	All fish expired	All fish expired	All fish expired
Significance				
% of change				

Effect of manganese :

Table (3) summerizes the data obtained for the activity of each of GOT, GPT (IU/min/l and acid (AP) and alkaline (Alp) phosphatases (kind and king Units/l) enzymes of the serum of Oreochromis niloticus after exposure to .8, 23.6 and 35.4 mg manganese/l (as manganous chloride) for 7 days versus the control group.

It is clear that the mean level of GOT activity of the serum of O. niloticus increased as the dose of manganese was increased. The mean level of activity of the control fish was 27.0±0.7 IU/min/l. This level was increased to 31.0±0.7, 34.0±0.4 and 37.3±0.3 IU/min/l after subjecting the fish groups to the low, intermediate and high manganese doses respectively. These increases revealed that they were dose dependent. All of which were statistically highly significant ($p<0.001$). This represented an increase percent over the mean level of serum GOT activity of the control group amounting to 14.8%, 25.9% and38% for the fish exposed to the low, intermediate and high manganese doses respectively.

Also the mean level of GPT activity of the serum of O. niloticus was markedly increased. The mean level of the enzyme activity was 9.0±0.40, 10.30±0.10, 13.60±0.30 and 19.10±0.20 IU/min/l for the control fish group and fish subjected to the low, intermediate and high doses of manganese respectively. This increase was 14.5% in case of fish subjected to the low dose, 51% in case of fish subjected to intermediate dose and 112% in case of fish subjected to high dose, respectively. Satistically all of these increases were highly significant at $P<0.05$ and $P<0.001$ levels respectively.

The mean level of acid (AP) phosphatase activity was markedly increased after subjecting the fish groups to the three doses of manganese as compared with that of the control group. The mean level of AP activity of the control group was 2.8±0.07 kind and king Units/l while that of fish subjected to the low, intermediate and high doses of manganese was 4.8±0.1, 5.5±0.08 and 6.5±0.1 kind and king Units/l respectively.This increase was highly significant ($p<0.001$) for all the three doses where it reached 71%, 96% and 132% of the mean control level for the low, intermediate and high doses respectively.

Also serum alkaline phosphatase (AlP) activity of Q. niloticus was markedly increased. The mean level of the enzyme activity was 21.0±0.4; 34.5±0.7, 39.6±0.5 and 44.6±0.7 kind and king Units/l for the control fish group and fish subjected to the low, intermediate and high doses of manganese respectively. This increase was 64% in case of fish treated with the low dose, 88% in case of fish subjected to the intermediate dose and 112% for those exposed to the high manganese dose respectively. Statistically all of these increases were highly significant at $p<0.001$ levels.

As it can be stated from table (3) that there was an increase of the mean level of activity of the four serum (GOT, GPT, AP and AlP) enzymes tested due to treatment with manganese as manganous chloride. However, the increases of serum AP and Alp activities were pronouncing than that of GOT and GPT activities.

Table (3): Mean and range values of serum glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) (IU/min/l) and acid (AP) and alkaline phosphatases (AlP) (kind and king units/l) activities of Oreochromis niloticus after exposure to three concentrations of manganese (as manganous chloride) for 7 days (7 animals were used per test).

	GOT	GPT	AP	AlP
Control Mean ± S.E. Range	27.0 ± 0.70 24.0 — 29.0	9.0 ± 0.40 7.0 — 10.0	2.80 ± 0.07 2.50 — 3.0	21.00 ± 0.40 20.0 — 22.0
Low dose Mean ± S.E. Range Significance % of change	31.0 ± 0.70 29.0 — 32.0 P < 0.02 + 14.8	10.30 ± 0.10 10.0 — 11.0 P < 0.05 + 14.5 %	4.80 ± 0.10 4.20 — 5.50 P < 0.01 + 71 %	34.5 ± 0.70 35.0 — 38.0 P < 0.001 + 64 %
Intermediate dose Mean ± S.E. Range Significance % of change	34.0 ± 0.40 33.0 — 35.0 P < 0.001 + 25.9	13.6 ± 0.30 13.0 — 15.0 P < 0.001 + 51 %	5.50 ± 0.08 5.30 — 5.80 P < 0.001 + 96 %	39.60 ± 0.50 38.0 — 42.0 P < 0.001 + 88 %
High dose Mean ± S.E. Range Significance % of change	37.30 ± 0.30 36.0 — 38.0 P < 0.001 + 38 %	19.10 ± 0.20 18.0 — 20.0 P < 0.001 + 112 %	6.50 ± 0.10 6.10 — 6.80 P < 0.001 + 132 %	44.60 ± 0.70 42.0 — 48.0 P < 0.001 + 112 %

Effect of zinc :

Table (4) shows the data obtained for the activity of each of serum glutamate oxaloacetate transaminase (GOT), glutamate pyruvate tronsaminase (GPT) (IU/min/l) and acid (AP) and alkaline (Alp) phosphatases (kind and king Units/l) enzymes of the fish; Oreochromis niloticus after exposure to 7.9 (low dose), 15.8 (intermediate dose) and 23.7 (high dose) mg/l of zinc (as zinc sulphate) for 7days as compared with that of the control group.

It is evident that serum GOT mean level of activity of O.niloticus of the control group was 27.0±0.7 IU/min/l where as those of fish subjected to 7.9, 15.8 and 23.7mg/l of zinc (as zinc sulphate) were 36.8±0.3, 40.0± 0.4 and 53.6±0.7 IU/min/l respectively. The differences between the mean level of GOT activity of the control group and that of the three different zinc levels treated groups were highly significant ($p<0.001$). The data declared an increase of the mean level of GOT activity reaching 36%,48% and 96% for the fish subjected to the low, intermediate and high doses respectively.

GPT mean level of activity of the serum of O.niloticus exposed to different levels of zinc for 7 days declared a marked increase also from that of the control fish. This increase was more or less dose dependent of zinc(Table 4). Statistically these increases of serum GPT activity of fish subjected to the low, intermediate and high doses were highly significant ($p<0.001$). These increases were 53%, 105% and 144% for the fish exposed to the low, intermediate and high zinc doses respectively.

Mean serum acid phosphatase activity of the Nile fish; O. niloticus was found to be markedly increased due to subjection of the fish to the three doses (low, intermediate and high) of zinc. The mean serum AP activity of the control fish proup was 2.8±0.07 kind and king Units/l which was increased to 5.4±0.1, 7.5±0.1 and 7.7±0.07 kind and king Units/l after subjecting the fish groups to low, intermediate and high zinc doses respectively. Statistical analysis showed that there was a highly significant increase ($p<0.001$) of serum AP activity due to treating fish with any of the three doses of zinc respectively. This represented 92%, 167% and 175% increase respectively (Table 4).

The table declared also that the mean level of serum alkaline (Alp) phosphatase activity was increased significantly ($p<0.001$) after subjecting the fish to low, intermediate and high zinc doses respectively. This increase reached 58%, 128%and 149% respectively over that of the control fish group. (Table 4).

As it can be seen from the previously cited data there was a marked increase of the mean level of activity of the serum enzymes tested (GOT,GPT,AP and AlP) due to zinc treatment at any of the three cocentration levels used.

Effect of Copper :

The mean values of each of glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) (IU/min/l) and acid (AP) and alkaline (AlP) phosphatases(kind and king Units/l) enzymes of the serum of the Nile fish; Oreochromis niloticus after subjection to three concentrations of copper (low 0.126mg/l, intermediate 0.252mg/l and high 00.278mg/l) as copper sulphate for 7 days as compared with those of the control group are shown in table (5).

It is clear that there was a marked increase of serum GOT activity with increasing copper dose. GOT mean activity of the control fish was 27±0.7 IU/min/l which increased to 31.3±0.3, 33.3±0.3 and 39.3±0.8 IU/min/l after subjecting the fish groups to low, intermediate and high copper doses respectively. This increase was statistically significant ($p< 0.01$, $p<0.002$ and $p<0.001$ respectively). It reached 15.9%, 23% and 45.5% in the order mentioned.

The same table depicts that the GPT mean level of activit increased also by increasing copper dose. This level was 9.6±0.3,13.3±0.1 and 15.0±0.2 IU/min/l for the three groups of fish respectively compared to 9.0±0.4 IU/min/l for the control fish group. Statistically the increase in case of low copper dose treated group was insignificant, while those of the other two doses were highly significant ($p<0.001$) and amounted to 6.6%, 47.7% and 66.6% respectively.

Similarly the mean acid phosphatase (AP) level of activity of the control group was 2.8±0.07 kind and king Units/l was increased to 3.6± 0.1, 5.3±0.1 and 6.2±0.08 kind and king Units/min/l for the fish groups treated with the low, intermediate and high copper doses respectively. These increases reached 28.5%, 89% and 121% over that of the mean control fish group.

Furthermore, serum alkaline phosphatase (AlP) activity increased also by 25%, 53.8% and 75% due to increasing copper dose respectively. This increase was significant ($p<0.01$) in case of the low level of copper while it was highly significant ($p<0.001$) in case of the intermediate and high doses of the same metal (Table 5).

As it can be seen in table (5) the three copper doses (low, intermediate and high) affeced markedly the four enzyme activities and mostly this effect was dose dependent. The effect of the high copper dose on AP and AlP activities was markedly pronouncing than on the other two enzymes.

Table (5): Mean and range values of serum glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) (IU/min/l) and acid (AP) and alkaline (AIP) phosphatases (kind and king Units/l) activities of Oreochromis niloticus after exposure to three concentrations of copper (as copper sulphate) for 7 days (7 animals were used per test).

	GOT	GPT	AP	AIP
Control				
Mean ± S.E.	27.0 ± 0.70	9.0 ± 0.40	2.80 ± 0.07	21.00 ± 0.40
Range	24.0 — 29.0	7.0 — 10.0	2.50 — 3.0	20.0 — 22.0
Low dose				
Mean ± S.E.	31.30 ± 0.30	9.60 0.30	3.60 ± 0.10	26.30 ± 0.70
Range	30.0 — 32.0	9.0 — 11.0	3.0 — 3.90	24.0 — 29.0
Significance	P < 0.01	insignificant	P < 0.001	P < 0.01
% of change	+ 15.9 %	+ 6.6 %	+ 28.5%	+ 25 %
Intermediate dose				
Mean ± S.E.	33.30 ± 0.30	13.3 ± 0.10	5.30 ± 0.10	32.30 ± 0.60
Range	32.0 — 34.0	13.0 — 14.0	5.0 — 5.80	31.0 — 35.0
Significance	P < 0.002	P < 0.001	P < 0.001	P < 0.001
% of change	+ 23 %	+ 47.7 %	+ 89 %	+ 53.8%
High dose				
Mean ± S.E.	39.30 ± 0.80	15.0 ± 0.20	6.20 ± 0.08	36.80 ± 0.70
Range	37.0 - 42.0	14.0 - 16.0	5.90 - 6.50	35.0 - 40.0
Significance	P < 0.001	P < 0.001	P < 0.001	P < 0.001
% of change	+ 45.5%	+ 66.6 %	+ 121 %	+ 75 %

Total Liver Protein:

The mean values of the total protein of the liver of the nile fish; Oreochromis niloticus after exposure to three levels of each of iron (as ferric chloride), manganese (as manganous chloride), zinc (as zinc sulphate) and copper (as copper sulphate) for 7 days are shown in table (6). As it can be depicted from the table the mean level of the total proteins of the liver of the control O. niloticus was 0.018 ± 0.003 g/g animal tissue whereas that for fishes subjected to the low, intermediate and high doses of iron was 0.043 ± 0.002 and 0.064 ± 0.002 g/g liver tissue respectively. Fishes subjected to the third high concentration (515.4 mg/l) of iron died within few hours from starting of the experiment. Statistical analysis showed that there was a highly significant increase ($P<0.01$ and $P < 0.001$) of the total liver protein due to subjecting the fish groups to the low and intermediate doses of iron containing water. These increases represented 138% and 255% over the mean control level respectively.

Table (6) depicts that the effect of manganese (as manganous chloride) on the total liver protein of O. niloticus was a marked increase. Fishes subjected to the three levels of manganese revealed a mean total liver protein of 0.023 ± 0.002, 0.038 ± 0.004 and 0.052 ± 0.003 g/g liver tissue for the three manganese treated fish groups respectively. Statistically all of these data were highly significantly increased ($P < 0.01$, $P<0.02$ and $P<0.002$) at the three manganese levels respectively. The increase reached 94%, 111% and 188% of the mean total liver protein content of the control fish for the low, intermediate and high manganese treated fish groups respectively.

Similarly total liver protein of fish groups treated with zinc as zinc sulphate was markedly increased. This increase was 83%, 150% and 233% for fish groups treated with water containing low, intermediate and high doses of zinc, respectively. It is worthy to mention that these increases were statistically significant ($P < 0.01$, $P < 0.02$ and $P < 0.002$) for the low, intermediate and high zinc treated fish respectively.

The mean total liver protein of fishes raised in normal water and water containing the three doses of copper (low, intermediate and high doses) as copper sulphate were 0.018 ± 0.003, 0.024 ± 0.0009, 0.032 ± 0.003 and 0.042 ± 0.008 g/g liver tissue respectively. It is clear that the total protein of liver O. niloticus treated with the low level of copper was insignificantly increased while that of fish subjected to either intermediate or high dose of copper was significantly increased ($P<0.05$). Furthermore these increases reached 33%, 77.7% and 133% over that of the mean control group.

It is clear that the four metals (iron, manganese, zinc and copper) had affected markedly total liver protein level. However, the effect of low doses of copper and zinc was less than that of iron, while the high doses used of copper, manganese and zinc induced detectable increase of liver protein synthesis.

Table (6): Mean values of total liver protein (g/g liver tissue) of Oreochromis niloticus after exposure to three levels of iron, manganese, zinc and copper for 7 days (7 animals were used per test).

	Iron	Manganese	Zinc	Copper
Control				
Mean ± S.E.	0.018 ± 0.003			
Range	0.001—0.037			
Low dose				
Mean ± S.E.	0.043 ± 0.002	0.035±0.001	0.033 ± 0.0004	0.024 ± 0.0009
Range	0.032—0.052	0.026—0.040	0.032—0.034	0.020—0.027
Significance	P < 0.01	P<0.01	P<0.01	in significnat
% of change	+ 138%	+ 94%	+ 83%	+ 33%
Intermediate dose				
Mean ± S.E.	0.064 ± 0.002	0.038 ± 0.004	0.045 ± 0.0005	0.032 ± 0.003
Range	0.058—0.072	0.029—0.063	0.043—0.047	0.021—0.045
Significance	P < 0.001	P < 0.02	P < 0.001	P < 0.05
% of change	+ 255%	+111%	+150%	+77.7%
High dose				
Mean ± S.E.		0.052 ± 0.003	0.060 ± 0.004	0.042 ± 0.008
Range	All fish expired	0.044—0.068	0.043—0.079	0.019—0.080
Significance		P < 0.002	P < 0.002	P < 0.05
% of change		+188%	+233%	+133%

Liver Enzyme Activities

1. **Effect of Iron**:

Applying fish groups to different doses of iron as ferric chloride for 7 days resulted in an inhibition of liver glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) (IU/g tissue/min) and acid and alkaline phosphatases (Kind and King Units/g tissue) enzymes of the fish; Oreochromis niloticus. Fish exposed to the high dose of iron as ferric chloride (515.4 mg/l) died within few hours of subjection to that metal salt dose (Table 7).

The mean level of liver GOT activity was 2.613 ± 0.07 IU/g tissue/min for the control fish while it was 0.834 ± 0.03 and 0.394 ± 0.02 IU/g tissue/min in case of fish subjected to 171.8 and 343.6 mg $FeCl_3$/l (low and intermediate) doses respectively. However, there was a significant decrease ($P < 0.001$) of the mean level of liver GOT activity between the control group and that of the group of fishes subjected to the low and intermediate iron levels. The percentage of decrease reached 68% and 84.9%, respectively (Table7).

It is clear from the table that the mean level of GPT activity of the liver of O. niloticus was 0.653 ± 0.04 IU/g tissue/min while that of the fish groups exposed to the low and intermediate iron doses was 0.432 ± 0.008 and 0.216 ± 0.01 IU/g tissue/min respectively. The data revealed a decrease of the mean level of GPT activity which was statistically highly significant ($P < 0.01$ and $P<0.001$) for both iron doses treated fish as compared with that of the control group. This decrease reached 33.8% and 66.9% respectively.

The mean level of liver acid phosphatase activity of O.niloticus of the control group was 0.452 ± 0.007 Kind and King Units/g tissue. This activity was pronouncly decreased to reach 0.075 ± 0.003 and 0.049 ± 0.001 Kind and King Units/g tissue after exposing the fish to the low and intermediate doses of iron respectively (Table 7). This decrease was highly significant ($P < 0.001$) and reached 83.4% and 89% of the mean control liver level respectively.

The mean level of liver alkaline phosphatase activity of O.niloticus was 2.154 ± 0.02, 0.748 ± 0.03 and 0.464 ± 0.01 Kind and King Units/g tissue for the control, low and intermediate iron doses respectively. Statistically these results revealed a highly significant decrease ($P < 0.001$) of the mean level of alkaline phosphatase activity in case of both of the low and intermediate doses of iron treated fish groups. The percentage of decrease of the enzyme activity reached 65% and 78.4% respectively of that of the control group.

It is obvious that, there was a decrease in the mean level of liver activity of all of the above mentioned enzymes diu to treatment with the two doses when compared to the control group. At the high dose of iron the fishes were expired within few hours from the beginning of the experiment. This inhibitory effect of iron was markedly noticed in the case of alkaline phosphatase and least in case of GPT.

Table (7): Mean and range levels of glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transminase (GPT) (IU/g liver tissue/min) and acid (AP) and alkaline (AlP) phosphatases (kind and king Units/g tissue) activities of the liver of Oreochromis niloticus after exposure to three levels of iron (as ferric chloride) for 7 days (7 animals were used per test).

	GOT	GPT	AP	AlP
Control				
Mean ± S.E.	2.613 ± 0.07	0.653±0.04	0.452±0.007	2.154±0.02
Range	2.459—2.989	0.546—0.833	0.429—0.483	2.065—2.247
Low dose				
Mean ± S.E.	0.834 ± 0.03	0.432±0.008	0.075 ± 0.003	0.748 ± 0.03
Range	0.733—0.934	0.412—0.465	0.059—0.088	0.610—0.921
Significance	P < 0.001	P<0.01	P<0.001	P<0.01
% of change	-68%	-33.8%	-83.4%	-65%
Intermediate dose				
Mean ± S.E.	0.394 ± 0.02	0.216 ± 0.01	0.049 ± 0.001	0.464 ± 0.01
Range	0.324—0.467	0.185—0.277	0.046—0.056	0.416—0.514
Significance	P < 0.001	P < 0.001	P < 0.001	P < 0.001
% of change	-84.9%	-66.9%	-89%	-78.4%
High dose				
Mean ± S.E.				
Range	All fish expired	All fish expired	All fish expired	All fish expired
Significance				
% of change				

2. **Effect of Manganese**:

The data shown in table (8) revealed that the mean level of activity of GOT of liver of O. niloticus of the control group was 2.613 ± 0.07 IU/g tissue/min while the values of fishes exposed to low, intermediate and high doses of manganese as manganous chloride were 1.304 ± 0.04, 0.812 ± 0.03 and 0.365 ± 0.02 IU/g tissue/min respectively. It is clear that the decrease recorded between the mean activity of liver GOT of the control and that of any of all of the manganese treated groups was highly significant ($P < 0.001$). These decreases of the enzyme activity attained 50%, 68.9% and 86% for the three metal doses, respectively.

It is evident from table (8) that the mean level of GPT activity of liver was 0.653 ± 0.04, 0.451 ± 0.01, 0.338 ± 0.02 and 0.152 ± 0.009 IU/g tissue/min for the control and low, intermediate and high manganese doses treated fish groups respectively. It is seen that the decrease of the mean level of GPT activity was significant ($P < 0.01$) in case of the first dose (low dose) while it was highly significant ($P < 0.001$) in case of the other two concentrations. This decrease reached 30.9%, 48% and 76.7% of the mean control value for the three manganese doses used respectively.

The mean level of acid phosphatase activity of the liver of O.niloticus of the control group was 0.452 ± 0.007 Kind and King Units/ g tissue while that of those fishes subjected to low, intermediate and high levels of manganese were 0.170 ± 0.006, 0.154 ± 0.004 and 0.083 ±

0.004 Kind and King Units/g tissue/min respectively. The data clearly show that the mean level of the liver enzyme activity decreased significantly ($P < 0.001$) on subjecting the fish to any of the three manganese levels. This decrease amounted to 62.3%, 65.9% and 81.6% for the three levels respectively.

Regarding to liver alkaline phosphatase mean activity of the control fish group it was 2.154 ± 0.02 Kind and King Units/g tissue, while that recorded for the three levels of manganese was 1.622 ± 0.03, 0.834 ± 0.02 and 0.740 ± 0.03 Kind and King Units/g tissue respectively. The data showed a marked decrease of the enzyme activity which was highly significant ($P< 0.001$) for all treated groups. The decrease of the mean level of liver alkaline phosphatase activity reached 24.6%, 61% and 65.6% for fishes subjected to low, intermediate and high levels of manganese, respectively.

As it can be seen from the previously cited data that there was a marked decrease of the mean level of activity of the four enzymes of the fish liver under investigation when they were exposed to the above mentioned concentrations as compared to that of the control group. However, such inhibition was considerably slight in case of liver GPT of the fish groups subjected to the low and intermediate manganese doses and liver alkaline phosphatase of the low metal treated fish.

Table (8): Mean and range levels of glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transminase (GPT) (IU/g liver tissue/min) and acid (AP) and alkaline (AlP) phosphatases (kind and king Units/g tissue) activities of the liver of Oreochromis niloticus after exposure to three levels of manganese (as manganous chloride) for 7 days (7 animals were used per test).

	GOT	GPT	AP	AlP
Control				
Mean ± S.E.	2.613 ± 0.07	0.653±0.04	0.452±0.007	2.154±0.02
Range	2.459—2.989	0.546—0.833	0.429—0.483	2.065—2.247
Low dose				
Mean ± S.E.	1.304 ± 0.04	0.451±0.01	0.170 ± 0.006	1.622 ± 0.03
Range	1.188—1.450	0.402—0.466	0.150—0.188	1.534—1.776
Significance	P < 0.001	P<0.01	P<0.001	P<0.001
% of change	-50%	-30.9%	-62.3%	-24.6%
Intermediate dose				
Mean ± S.E.	0.812 ± 0.03	0.338 ± 0.02	0.154 ± 0.004	0.834 ± 0.02
Range	0.666—0.896	0.266—0.413	0.140—0.173	0.738—0.896
Significance	P < 0.001	P < 0.002	P < 0.001	P < 0.001
% of change	-68.9%	-48%	-65.9%	-61%
High dose				
Mean ± S.E.	0.365±0.02	0.152±0.009	0.083±0.04	0.740±0.03
Range	0.307—0.510	0.150—0.157	0.068—0.097	0.653—0.842
Significance	P<0.001	P<0.001	P<0.001	P<0.001
% of change	-86%	-76.7%	-81.6%%	-65.6%

3. **Effect of Zinc**:

Application of zinc as zinc sulphate at the three chosen concentrations to the fish; O. niloticus for one week induced a marked reduction of liver GOT activity. From table (9), it is clear that the mean level of GOT activity of the liver of the control group was 2.613 ± 0.07 IU/g tissue/min, while that of fishes exposed to the low, intermediate and high doses of zinc was 0.865 ± 0.02, 0.692 ± 0.02 and 0.550 ± 0.03 IU/g tissue/min respectively. Analysis of the data presented in table (9) revealed that, there was a significant decrease ($P < 0.001$) of the mean level of liver GOT activity of the fish groups subjected to the three zinc levels as compared with that of the control group. The decrease was 66.8%, 73.5% and 78.9% of the mean control value respectively.

The mean level of liver GPT activity of O. niloticus of the control group it was 0.653 ± 0.04 IU/g tissue/min while that of fishes exposed to the low, intermediate and high zinc levels was 0.535 ± 0.02, 0.215 ± 0.01 and 0.132 ± 0.009 IU/g tissue/min in the order mentioned. Statistically, it is clear that there was a moderate significant decrease ($P < 0.05$) of the mean level of liver GPT activity of the fish groups subjected to the low level of zinc, but a marked decrease ($P < 0.001$) of the enzyme activity was recorded after application of the two higher zinc levels. These decreases were 18%, 67% and 79.7% of the mean control level for those fishes treated with low, intermediate and high doses respectively.

A pronouncing decrease of liver acid phosphatase activity ($P < 0.001$) was found when O. niloticus was subjected to any of the three zinc

concentrations used. The mean level of activity was 0.170 ± 0.004, 0.112 ± 0.005 and 0.083 ± 0.002 Kind and King Units/g tissue for the fishes treated with the three doses of zinc, respectively compared to 0.452 ± 0.007 Kind and King Units/g tissue for that of the control group (Table 9). This decrease reached 62%, 75.5% and 81.6% of the mean level of the control value for the three concentrations of zinc respectively.

Table (9) shows also that the mean level of alkaline phosphatase activity of the liver of the control Q.niloticus group was 2.154 ± 0.02 Kind and King Units/g tissue, yet that of the three groups treated with zinc as zinc sulphate for one week was 0.934 ± 0.008, 0.653 ± 0.03 and 0.434 ± 0.02 Kind and King Units/g tissue respectively. It is clear that there was a highly significant ($P < 0.001$) decrease of liver alkaline phosphatase activity due to application of the three levels of zinc as compared with that of the control group. The data declared a decrease of 56.6%, 69% and 79.8% of the mean control value for fishes treated with the low, intermediate and high doses of zinc respectively.

Considering the previous data it is evident that there was a marked decrease of the mean level of activity of each of liver GOT, GPT and acid and alkaline phosphatases activities due to zinc treatment. However, the low dose caused a moderate but significant decrease of liver GPT activity as compared to the effect of the same dose on the other enzymes.

Table (9): Mean and range levels of glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transminase (GPT) (IU/g liver tissue/min) and acid (AP) and alkaline (AlP) phosphatases (kind and king Units/g tissue/min) activities of the liver of Oreochromis niloticus after exposure to three levels of zinc (as zinc sulphate) for 7 days (7 animals were used per test).

	GOT	GPT	AP	AlP
Control				
Mean ± S.E.	2.613 ± 0.07	0.653±0.04	0.452±0.007	2.154±0.02
Range	2.459—2.989	0.546—0.833	0.429—0.483	2.065—2.247
Low dose				
Mean ± S.E.	0.865 ± 0.02	0.535±0.02	0.170 ± 0.004	0.934 ± 0.008
Range	0.800—0.943	0.471—0.650	0.154—0.183	0.900—0.972
Significance	P < 0.001	P<0.05	P<0.001	P<0.001
% of change	-66.8%	-18%	-62%	-56.6%
Intermediate dose				
Mean ± S.E.	0.692 ± 0.02	0.215 ± 0.01	0.112 ± 0.005	0.653 ± 0.03
Range	0.593—0.744	0.182—0.281	0.078—0.094	0.509—0.751
Significance	P < 0.001	P < 0.001	P < 0.001	P < 0.001
% of change	-73.5%	-67%	-75.5%	-69%
High dose				
Mean ± S.E.	0.550±0.03	0.132±0.009	0.083±0.002	0.434±0.02
Range	0.458—0.610	0.100—0.158	0.078—0.094	0.321—0.516
Significance	P<0.001	P<0.001	P<0.001	P<0.001
% of change	-78.9%	-79.7%	-81.6%	-79.8%

4. **Effect of Copper**:

Table (10) shows the activity of each of glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) (IU/g tissue/min) and acid and alkaline phosphatases (Kind and King Units/g tissue) enzymes of the liver of Oreochromis niloticus after exposure to three concentrations of copper sulphate (0.126 mg/l low dose, 0.252 mg/l intermediate dose and 0.378 mg/l high dose) for 7 days.

It is clear from the table that the mean level of liver GOT activity was 2.613 ± 0.07, 1.451 ± 0.07, 0.608 ± 0.05 and 0.351 ± 0.05 IU/g tissue/min for the control fish group and fishes subjected to low, intermediate and high doses of copper respectively. It is evident that copper treatment induced a marked decrease of liver GOT activity and this decrease was highly significant for all copper doses used ($P < 0.001$). The data declared a decrease of the mean level of GOT activity reaching 44.4%, 76.7% and 86.5% of the mean control enzyme activity for those fish raised for one week in the low, intermediate and high copper levels respectively.

Furthermore, copper as copper sulphate induced also a significant decrease of liver GPT activity of fish raised in any of the three concentrations used. However, these decreases were not as deep as those found in case of GOT activity. The mean liver activity of GPT was 0.653 ± 0.04 IU/g tissue/min for the control whereas it was 0.494 ± 0.03, ($P < 0.05$), 0.213 ± 0.01 ($P < 0.001$) and 0.170 ± 0.01, ($P < 0.001$) IU/g tissue/min for those fish groups subjected to the low, intermediate and

high doses of copper for one week respectively (Table 10). These decreases of the mean level of GPT activity reached 24.3%, 67.3% and 73.9% of the mean control level for the three concentrations used respectively.

The mean level of acid phosphatase activity of the liver of O. niloticus of the control fish group was 0.452 ± 0.007 Kind and King Units/g tissue, while that for those fishes subjected to the low, intermediate and high copper levels was 0.251 ± 0.004, 0.173 ± 0.004 and 0.097 ± 0.005 Kind and King Units/g tissue respectively. Statistically, it could be observed that copper treatment resulted in a marked decrease of the enzyme activity due to all doses used. This decrease was significant ($P < 0.001$) for the results obtained after the three concentrations application. These decreases reached 44.4%, 61.7% and 78.5% for the low, intermediate and high copper doses used as compared with that of the mean control value.

Liver alkaline phosphatase mean activity of the fish groups was affected also by copper application. The mean activity of the control group was 2.154 ± 0.02 Kind and King Units/g tissue while it was 1.827 ± 0.05, 0.763 ± 0.06 and 0.513 ± 0.07 Kind and King Units/g tissue after application of the three levels of copper respectively. This indicated a statistically significant ($P < 0.01$) decrease in case of the low copper level and a highly significant ($P < 0.001$) one in case of the two higher copper levels. This decrease reached 15%, 64.5% and 76% of the mean control value after subjecting the fish to the three copper concentrations respectively (Table 10).

It can be seen from the previous results that there was a general decrease of all liver enzyme activities under investigation due to application of the three copper levels. However, it is clear that the effect induced by the low copper level was least in case of alkaline phosphatase and GPT. Doubling copper concentration on the other hand, sharply inhibited the activities of all enzymes tested.

Table (10): Mean and range levels of glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transminase (GPT) (IU/g liver tissue/min) and acid (AP) and alkaline (AlP) phosphatases (kind and king Units/g tissue) activities of the liver of Oreochromis niloticus after exposure to three levels of copper (as copper sulphate) for 7 days (7 animals were used per test).

	GOT	GPT	AP	AlP
Control				
Mean ± S.E.	2.613 ± 0.07	0.653±0.04	0.452±0.007	2.154±0.02
Range	2.459—2.989	0.546—0.833	0.429—0.483	2.065—2.247
Low dose				
Mean ± S.E.	1.451 ± 0.07	0.494±0.03	0.251 ± 0.004	1.827 ± 0.05
Range	1.214—1.784	0.363—0.582	0.240—0.272	1.636—1.964
Significance	P < 0.001	P<0.05	P<0.001	P<0.01
% of change	-44.4%	-24.3%	-44.4%	-15%
Intermediate dose				
Mean ± S.E.	0.608 ± 0.05	0.213 ± 0.01	0.173 ± 0.004	0.763 ± 0.06
Range	0.440—0.724	0.158—0.258	0.160—0.187	0.582—0.942
Significance	P < 0.001	P < 0.001	P < 0.001	P < 0.001
% of change	-76.7%	-67.3%	-61.7%	-64.5%
High dose				
Mean ± S.E.	0.351±0.05	0.170±0.01	0.097±0.005	0.513±0.07
Range	0.247—0.640	0.114—0.200	0.075—0.119	0.346—0.852
Significance	P<0.001	P<0.001	P<0.001	P<0.001
% of change	-86.5%	-73.9%	-78.5%	-76%

Similarly the mean level of total brain protein of O. niloticus treated with copper (as copper sulphate) was markedly increased. This increase was 42.8%, 61.9% and 90% for fish groups treated with water containg low, intemediate and high doses of copper respectively. These increases were statistically highly significant ($P<0.001$) for fish groups treated with water containg low, intermediate and high doses of copper treated groups respectively.

It is clear that applying the four metals (iron, manganese, zinc and copper) with different doses to fish groups resulted in an increase of the mean total brain protein of the Nile fish; O.niloticus.

Table (11): Mean values of total brain protein (g / g brain tissue) of Oreochromis niloticus after exposure to three doses of iron, manganese, zinc and copper (as salts) for 7 days (7 animals were used per test).

	Control	Iron	Manganese	Zinc	Copper
Low dose					
Mean ± S.E.	0.021 ± 0.0003	0.036 ± 0.001	0.038 ± 0.0009	0.037 ± 0.001	0.030 ± 0.0005
Range	0.020 — 0.023	0.031 — 0.039	0.036— 0.042	0.030 — 0.042	0.028 — 0.032
Significance		P<0.001	P<0.001	P<0.001	P<0.001
% of change		+ 71%	+80.9%	+76%	+ 42.8%
Intermediate dose					
Mean ± S.E.		0.039±0.0005	0.039±0.0003	0.39±0.0006	0.034 ± 0.0006
Range		0.038—0.042	0.038—0.040	0.037—0.040	0.033 — 0.037
Significance		P<0.001	P<0.001	P<0.001	P<0.001
% of change		+85%	+85.7%	+95%	+61.9%
High dose			0.041 ± 0.00043	0.041±0.0008	0.040±0.0003
Mean ± S.E.			0.040— 0.043	0.038—0.044	0.040—0.042
Range		All fish expired	P<0.001	P<0.001	P<0.001
Significance			+95%	+85.7%	+90%
% of change					

Brain Enzyme Activities:

Effect of individual metal:

A set of experiments was carried out in which the individual metal solutions were applied on the fish at low (171.8 mg $Fecl_3$/l, 11.8 mg $Mncl_2$ /l, 7.9 mg $ZnSo_4$/l and 0.126 mg $CuSo_4$/l). Intermidiate (343.6 mg $FeCl_3$/l , 23.6 mg $MnCl_2$/l, 15.8mg $Znso_4$/l and 0.252 mg $CuSo_4$/l) and high (515.4 mg $Fecl_3$/l, 35.4 mg $MnCl_2$/l, 23.7 mg $ZnSo_4$/l and 0.378 mg $CuSo_4$/l) doses. The first one (low dose) was equivalent to that analyzed spectrophoto-metrically of the Nile water 2 meters a way from the shore. The other concentrations were doublicates (intermediate dose) and tripplicates (high dose) of the salt ion concentrations. In each test 7 fishes were reared in each individual metal level for 7 days.

1- Effect of Iron :

It was noticed that at the high iron level all the fishes died within few hours of application of the metal. Table (12) shows the data obtained for the activity of each of glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) (IU/g/tissue/ min), and acid and alkaline phosphatases (kind and king units/ g tissue) enzymes of the brain extracts of Oreochromis niloticus after exposure to 171.8, 343.6 and 515.4 mg iron/l of as ferric chloride as compared to the control fish.

It is evident that, the mean level of GOT activity of the brain of the control of O.niloticus was 2.540±0.10 IU/g tissue/ min and that

for fishes raised in the low iron dose for one week was 0.713±0.07 IU/g tissue/min. while that of those kept in the intermediate dose was 0.645± 0.07 IU/g tissue/ min . Statistically, the decrease of the mean level of GOT activity was highly significant ($p<0.001$) for both of the low and intermediate doses treated groups as compared to that of the control one. This decrease amounted to 71.9% in case of fishes treated with the low dose and 74.6% for those exposed to the intermediate dose which indicate a dose dependent decrease of the enzyme activity.

It is clear also that, the mean level of GPT activity of the brain of fish exposed to iron showed the same pattern of decrease, where the mean level of enzyme activity of the control was 0.985±0.05 IU/g tissue/ min which decreased to 0.408±05 and 0.278±0.03 IU/g tisue/min after exposing the fish to the low and intermediate doses respectively (Table12). The decrease of the mean level of GPT activity was highly signficant ($p<0.002$ and $p<0.001$) for the low and intermediate iron doses recpectively .It reached 58.5% and 71.7% of the value of the control respectively.

Furthermore , the mean level of acid phosphatase activity of the brain of the control fish group was 0.695±0.06 kind and king Units/g tissue, while that of fishes raised in the low dose of iron was 0.209±0.01 kind and king Units/g tissue and that of the fish exposed to the intermediate dose was 0.054 0.01 kind and king Units/g tissue. It is evident that the decrease of the mean level of acid phosphatase activity was highly significant ($P<0.002$),($P<0.001$) in both treated groups. This

decrease reached about 69.9% in case of fish exposed to the low dose and about 92.2% in case of fish exposed to intermediate dose.

The mean level of alkaline phosphatase activity of the brain of the control fish was 1.587 0.10 kind and king Units/ g tissue, while that of fishes exposed to the low and intermediate doses of iron was 0.614± 0.06 and 0.354±0.05 kind and king Units/ g tissue respectively. Statistical analysis revealed that the decrease of the mean level of alkaline phosphatase activity was highly significant ($P<0.002$) and ($P<0.001$) for the low and intermediate doses respectively . This decrease was about 61.3% in the case of the low dose exposed fish and about 77.6% in case of the intermediate dose exposed ones.

It can be seen from the previously cited data that there was a decrease in the mean level of activity of all of above mentioned enzyme activities of the brain of fishes subjected to the two doses of iron (as ferric chloride). This decrease was more deeper with increased dose of iron. However, fish subjected to the third dose (high dose) died within few hours of treatment with that metal salt level.

Table (12): Mean and range values of glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) (IU/g tissue/min) acid (AP) and alkaline (AlP) phosphatases (Kind and King Units/g tissue) activities of the brain of Oreochromis niloticus after exposure to three concentration levels of iron (as ferric chloride) for 7 days (7 animals were used per test).

	GOT	GPT	AP	AlP
Control				
Mean ± S.E.	2.540 ± 0.10	0.985 ± 0.05	0.695 ± 0.06	1.587 ± 0.1
Range	2.150—2.828	0.526—0.930	0.526—0.930	1.313—1.935
Low dose				
Mean ± S.E.	0.713 ± 0.07	0.408±0.05	0.209 ± 0.01	0.614 ± 0.06
Range	0.487—0.961	0.263—0.625	0.168—0.265	0.392—0.865
Significance	P < 0.001	P<0.002	P<0.002	P<0.002
% of change	- 71.9%	- 58.5 %	- 69.9 %	- 61.3 %
Intermediate dose				
Mean ± S.E.	0.645 ± 0.07	0.278 ± 0.03	0.054 ± 0.01	0.354 ± 0.05
Range	0.427—0.900	0.178—0.404	0.018—0.081	0.178—0.598
Significance	P < 0.001	P < 0.001	P < 0.001	P < 0.001
% of change	- 74.6 %	- 71.7 %	- 92.2 %	- 77.6%
High dose				
Mean ± S.E.				
Range	All fish expired	All fish expired	All fish expired	All fish expired
Significance				
% of change				

2- Effect of Manganese :

The data presented in table (13) depict the mean activity of each of glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) as (IU/ g fresh tissue / min) and acid and alkaline phosphatases (kind and king units/g fresh tissue) enzymes of the brain extracts of Oreochromis niloticus after exposing the fish groups to three levels of manganese (11.8, 23.6 and 35.4 mg $MnCl_2$/l) as manganese chloride for 7 days as compared to that of the control group.

It is evident from the table that GOT activity of the brain showed a steady decrease. The mean value of the control group was 2.540±0.10 IU/ g tissue/ min , wehreas that for those fish raised in low manganese level was 0.685±0.04 IU/ g tissue/min. That of fish subjected to the intermediate dose was 0.557±0.04 IU/g tissue/min and that of those exposed to the high dose was 0.468±0.01 IU/g tissue/min. Statistical analysis showed that the difference in the mean level of the control and each of the metal exposed group of fish to be highly significant ($p< 0.001$). Thus the mean level of GOT activity was decreased by about 73%, 78% and 81.5% in case of fish groups subjected to the low , intermediate and high doses of manganese respectively as compared with that of the control group.

With regards to the mean level of GPT activity of the brain of O.niloticus it was 0.985±0.0.05, 0.400±0.03, 0.245±0.02 and 0.145± 0.02 IU/g tissue/ min of the control group and those fishes exposed to the low, intermediate and high levels of manganese. The data declared

marked decreases of the mean GPT activity which were highly signifcant (P<0.001) for all of the three doses used as compared with that of the control group. The decrease of the mean level of GPT activity reached about 59% , 75% and 85% for the low, intermediate and high doses of manganese treated fish groups, respectively.

The mean activity of acid phosphatase of the brain of the control fish was 0.695± 0.06 king and king Units/ g tissue while that recorded after one week of subjecting the fish to the low, intermediate and high doses of manganese (as manganous chloride)was 0.512± 0.02, 0.119±0.003, and 0.072±0.007 kind and king Units /g tissue respectively. Statistically these results declare that there was a decrease of the mean level of acid phosphatase activity. This difference was significant (P< 0.05) in case of the low dose treated fish and highly significant (P>0.001) in case of the two higher doses treated fish groups. The decrease of the enzyme activity reached about 26% , 82.8% and 89.6% of the mean control value for the low, intermediate and high manganese treated fish groups respectively.

Table (13) reveals that the mean level of alkaline phosphatase of the brain of the control fish group was 1.587±0.10 kind and king Units/g tissue. It was 1.386 ±0.05, 1.107±0.02 and 0.646±0.07 kind and king Units/g tissue for those fishes subjected to the low, intermediate and high doses of manganese respectively . Statistical analysis showed that the difference found due to the low manganese dose was not significant. However, a significant (P<0.01) and a highly significant (P<0.002)

decreases were evident after subjection the fish to the intermediate and high doses of manganese respectively. The decrease of brain alkaline phosphatase activity was 12.6%, 30% and 59% of the mean control value for the low, intermediate and high manganese treated fishes respectively.

As it can be seen from the previously recorded observations there was a decrease of the mean level of activity of GOT, GPT and acid and alkaline phosphatases activities of the brain of fishes subjected to the three doses of manganese as manganous chloride. These decreases of the different enzyme activities were dose dependent. It is clear also that manganese effects were slightly lesser than the other metals tested on one hand and this effect was least on brain alkaline phosphatase on the other hand.

O.niloticus similarly was decreased due to fish subjection to all of zinc doses used. The mean activity of the control fish of brain acid phosphatase was 0.695±0.06 kind and king Units/g tissue. That of fish subjected to the low, intermediate and high doses of zinc was 0.499± 0.008, 0.254±0.01 and 0.159±0.007 kind and king Units/g tissue respectively. The decrease of the mean enzyme activily due to the low and intermediate doses was significant ($p<0.05$, $p<0.002$), yet it was highly significant ($P<0.001$) in case of the high dose of zinc treatment. This represented 28%, 63.4% and 77% decreases of brain acid phosphatase activity for the three doses respectively.

On the other hand, the mean alkaline phosphatase activity of the brain of fishes treated with the three doses was markedly decreased. The mean level of the enzyme activity of the control fish was 1.587±0.10 kind and king Units/g tissue, while at the low, dose of zinc it was 0.587± 0.05 kind and king Units/g tissue recording about 63% decrease from the mean control value. The mean enzyme activity of the brain of fish treated with the intermediate dose was 0.446±0.08 kind and king Units/g tissue with a decrease of 71.8%, while it was 0.293±0.04 kind and king Units/g tissue in case of fish treated with the high dose recording a decrease of 81.5%. Statistically all of these decreases were highly significant ($p<0.001$).

As it can be stated from the previous observations that there was a general decrease of the mean level of activity of the four enzymes tested due to treatment with zinc. However, the decreased fish brain GOT and alkaline phosphatase activities were more prominent and highly sounding especially on using the low dose of zinc than in the other two enzymes (Table14).

Table (14): Mean and range values of glutamate oxaloacetate transminase (GOT), glutamate pyruvate transaminase (GPT) (IU/g tissue/min) acid (AP) and alkaline (AlP) phosphatases (Kind and King Units/g tissue) activities of the brain of Oreochromis niloticus after exposure to three concentration levels of zinc (as zinc sulphate) for 7 days (7 animals were used per test).

	GOT	GPT	AP	AlP
Control				
Mean ± S.E.	2.540 ± 0.10	0.985 ± 0.05	0.692 ± 0.06	1.587 ± 0.10
Range	2.150— 2.828	0.808 — 1.162	0.526 — 0.930	1.157 — 1.935
Low dose				
Mean ± S.E.	1.290 ±0 .05	0.781±0.03	0.499 ± 0.008	0.587 ± 0.05
Range	1.150 — 1.454	0.673 — 0.882	0.471 — 0.539	0.394 — 0.700
Significance	P < 0.001	P<0.05	P<0.05	P<0.001
% of change	- 49%	- 20.7 %	- 28 %	- 63 %
Intermediate dose				
Mean ± S.E.	0.764± 0.04	0.325 ± 0.01	0.254 ± 0.01	0.446 ± 0.08
Range	0.614 — 0.892	0.303 — 0.421	0.216 — 0.305	0.202 — 0.736
Significance	P < 0.001	P < 0.001	P < 0.002	P < 0.001
% of change	- 69.9%	- 67 %	- 63.4 %	- 71.8%
High dose				
Mean ± S.E.	0.491 ± 0.003	0.256 ± 0.02	0.159 ± 0.007	0.293 ± 0.04
Range	0.485 - 0.500	0.217 - 0.329	0.131 - 0.187	0.217 - 0.444
Significance	P < 0.001	P < 0.001	P < 0.001	P < 0.001
% of change	- 80.6%	- 74 %	- 77 %	- 81.5 %

0.06 kind and king Units/g tissue respectively. Statistical analysis showed that the difference between the mean level of alkaline phosphatase activity of the control and copper sulphate treated fish was highly significant (Table 15).This decrease in the mean level of alkaline phosphatase activity reached about 25%, 48% and 63.5% in the case of low, intermediate and high doses treated groups respectively which declare that the inhibition of alkaline phosphatase induced by copper was dose dependent.

As it can be seen from the previously cited data the mean level of activities of all of the above mentioned enzymes (GOT, GPT, acid and alkaline phosphatases) of the brain of Oreochromis niloticus were inhibited markedly due to copper sulphate treatment.

Table (15): Mean and range values of glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) (IU/g tissue/min), acid (AP) and alkaline (AlP) phosphatases (kind and king Units/g tissue) activities of the brain of Oreochromis niloticus after exposure to three concentration levels of copper (as copper sulphate) for 7 days (7 animals were used per test).

	GOT	GPT	AP	AlP
Control				
Mean ± S.E.	2.540 ± 0.10	0.985 ± 0.05	0.695 ± 0.06	1.587 ± 0.10
Range	2.150 — 2.828	0.526 — 0.930	0.526 — 0.930	1.157 — 1.935
Low dose				
Mean ± S.E.	1.551 ± 0.06	0.707 ± 0.04	0.536 ± 0.05	1.184 ± 0.05
Range	1.368 — 1.769	0.517 — 0.796	0.366 — 0.736	1.009 — 1.382
Significance	P < 0.002	P < 0.02	insignificant	P < 0.05
% of change	- 38.9%	- 28%	-22.8%	- 25%
Intermediate dose				
Mean ± S.E.	0.756 ± 0.06	0.443 ± 0.06	0.159 ± 0.007	0.823 ± 0.03
Range	0.571 — 0.970	0.202 — 0.700	0.126 — 0.181	0.679 — 0.909
Significance	P < 0.001	P < 0.01	P < 0.001	P < 0.002
% of change	-70%	-55%	- 77%	- 48%
High dose				
Mean ± S.E.	0.695 ± 0.06	0.428 ± 0.05	0.064 ± 0.004	0.578 ± 0.06
Range	0.500 — 0.909	0.265 — 0.600	0.050 — 0.080	0.357 — 0.796
Significance	P < 0.001	P < 0.002	P < 0.001	P < 0.001
% of change	-72.7%	-56.5%	-90.7%	-63.5%

HAEMATOLOGY

Effect of Iron:

The data presented in table (16) depicts the mean values of red blood cell count (x 10^6), haemoglobin level (Hb g/100 ml) and haematocrit value (HC%) of the blood of Oreochromis niloticus after exposure to iron (as ferric chloride) for 7days as compared with that of the control fish group. It is clear that the mean RBC count of O. niloticus subjected to the low and intermediate doses of iron was 4.11±0.03 and 4.40±0.04 million/cmm respectively compared with 2.50±0.20 million/ cmm in case of the control group. Statistically the increase of RBC was highly significant ($p<0.002$ and $p<0.001$) for both groups and the percentage of increase was 64% and 76% for the low and interdediate iron doses exposed fish respectively (Table 16). Fish exposed to the high iron dose (515.4 mg/l) were expired after few hours.

The mean blood Hb level of the control fish group was 8.51/±0.13 g/100 ml while that for those groups of fishes exposed to the low and intermediate doses of iron was 10.0±0.23 and 10.50±0.20 g/100 ml. Statistical analysis showed that there was a siginificant ($p<0.01$) and highly significant ($p<0.002$) increase in mean Hb level of the low and intermediate treated doses fish respectively. The increase reached 17.5% and 23% respectively.

The mean HC value of O. niloticus subjected to iron for 7days was markedly increased. This increase was statistically significant ($p<0.01$

and $p<0.001$) respectively for the low and intermediate dose treated groups. The percentage of increase reached 19.5% for the low iron dose and 26% for the intermediate one treated fish.

It is obvious from the table that there was a marked increase of the mean values of the blood parameters tested (RBC, Hb and HC) after subjecting the Nile fish; Oreochormis niloticus to the two iron doses (low and intermediate). The high iron dose was found to be lethal to the fish.

Table (16): Mean and range values of red blood cell count ($x10^6$), haemoglobin level (Hb g/100 ml) and haematocrit value (HC%) of the blood of Oreochromis niloticus after esposure to three concentration levels of iron (as ferric chloride) for 7 days (7 animals were used per test).

	RBC	Hb	HC
Control			
Mean ± S.E.	2.50 ± 0.20	8.51 ± 0.13	23.0 ± 0.50
Range	2.0 — 3.20	8.0 — 8.90	21.0 — 25.0
Low dose			
Mean ± S.E.	4.11 ± 0.03	10.0 ± 0.23	27.50 ± 0.50
Range	4.0 — 4.20	9.0 — 11.0	26.0 — 29.0
Significance	P < 0.002	P < 0.01	P < 0.01
% of change	+ 64%	+ 17.5 %	+ 19.5 %
Intermediate dose			
Mean ± S.E.	4.40 ± 0.04	10.50 ± 0.20	29.0 ± 0.30
Range	4.20 — 4.50	10.0 — 11.0	28.0 — 30.0
Significance	P<0.001	P < 0.002	P < 0.001
% of change	+ 76 %	+ 23 %	+ 26 %
High dose			
Mean ± S.E.			
Range	All fish expired	All fish expired	All fish expired
Significance			
% of change			

Effect of Manganese:

The mean and range values of red blood cell count RBC (x 10^6), haemoglobin content (Hb g/100 ml) and haematocrit value (HC%) after subjecting Oreochromis niloticus to three doses of manganese (as manganous chloride) for 7 days versus the control fish group are summerized in table (17). It is clear that there was a marked increase of the three blood parameters (RBC, Hb and HC) tested as a result of manganese treatment as compared with that of the control fish group.

The red blood cell count of O. niloticus increased by 24.8%, 26% and 64% as a result of manganese (low, intermediate and high doses) treatment (Table 17). This increase was significant for the three manganese doses respectively.

The mean Hb level of O. niloticus of the control group was 8.51± 0.13 g/100 ml. This value increaed to 9.53±0.12, 10.60±0.08 and 11.58± 0.11 g/100 ml after treating the fish groups with the low, intermediate and high manganese doses (as manganous chloride). This increase was statistically significant ($p<0.01$) after treatment with the low dose of manganese and highly significant ($p<0.001$) after treatment with the intermediate and high doses respectively. It reached 11.9%, 24.5% and 36% for the low, intermediate and high doses respectively. (Table 17).

The table dipects also that the mean HC value of the control group and those groups subjected to low, intermedate and high manganese doses

was 23.0±0.5, 24.30±0.30, 26.60±0.50 and 27.30±0.60% respectively. Statistical analysis showed that the increase induced by low manganese dose was insignificant while it was significant ($p<0.01$) for the other two doses. The percentage of increase reached 5.6%, 15.6% and 18.6% for the three doses respectively.

It can be noticed that manganese treatment caused an increase of all the blood parameters (RBC, Hb and HC) of Q. niloticus tested in a doses dependent manner.

Table (17): Mean and range values of red blood cell count (RBC) ($x10^6$), haemoglobin level (Hb g/100 ml) and haematocrit value (HC%) of Oreschromis niloticus after exposure to three concentration levels of manganese (as manganous chloride) for 7 days (7 animals were used per test).

	RBC	Hb	HC
Control			
Mean ± S.E.	2.50 ± 0.20	8.51 ± 0.13	23.0 ± 0.50
Range	2.0 — 3.20	8.0 — 8.90	21.0 — 25.0
Low dose			
Mean ± S.E.	3.12 ± 0.03	9.53 ± 0.12	24.30 ± 0.30
Range	3.0 — 3.22	9.0 — 9.90	23.0 — 25.0
Significance	P < 0.005	P < 0.01	in significant
% of change	+ 24.8%	+ 11.9 %	+ 5.6 %
Intermediate dose			
Mean ± S.E.	3.15 ± 0.08	10.60 ± 0.08	26.60 ± 0.50
Range	3.0 — 3.60	10.30 — 10.90	25.0 — 29.0
Significance	P < 0.05	P < 0.001	P < 0.01
% of change	+ 26 %	+ 24.5 %	+ 15.6 %
High dose			
Mean ± S.E.	4.10 ± 0.04	11.58 ± 0.11	27.30 ± 0.60
Range	4.0 - 4.30	11.0 - 11.90	25.0 - 29.0
Significance	P < 0.002	P <0.001	P < 0.01
% of change	+ 64 %	+ 36 %	+ 18.6 %

Effect of Zinc:

Treating Oreochromis niloticus with the three levels of zinc (7.9, 15.8, 23.7 mg/l) as zinc sulphate for 7 days yeilded a general decrease of the mean value of each of red blood cell count, haemoglobin and haematocrit value.(Table 18)

Zinc treatment caused a progressive decrease of RBC count. This count was 1.90±0.21, 1.50±0.16 and 1.10±0.03 $x10^6$ for the low, intermediate and high doses respectively. The decrease was statistically insignificant in case of the low dose treated fish, while it was significant ($p<0.02$ and $p<0.01$) for the other two higher doses with a decrease of 40% and 56% for the intermediate and high zinc doses respectively (Table 18).

The mean Hb level of the control fish group was 8.51±0.13 g/100 ml while that of those fishes raised in low, intermediate and high doses of zinc containing water was 5.71%±0.16, 4.30±0.27 and 3.63±0.36 g/100 ml respectively. Statistical analysis declared that there was a highly significant ($p<0.001$) decrease of the mean Hb level as a result of the three zinc doses treatment. This decrease was 32%, 49% and 57% due to treatment with low, intermediate and high zinc doses respectively.

Zinc administration caused also a significant ($p<0.002$) decrease of HC value of the low dose and highly significant ones ($P< 0.001$) of the intermediate and high doses respectively. The mean HC value of the control group was 23.0±0.50, while it was 16.50±0.60, 14.0±0.40 and

9.50±20 after subjecting the fish to low, intermediate and high zinc doses respectively. These decreases reached 28%, 39% and 58.6% for the three doses respectively.

It is evident from the data that the three doses of zinc (as zinc sulphate) affected each of RBC, Hb and HC markedly causing a progressive decrease in all of the three blood parameters with increased zinc level.

Table (18): Mean and range values of red blood cell count (RBC) ($x10^6$), haemoglobin level (Hb) (g/100 ml) and haematocrit value (HC%) of the blood of Oreochromis niloticus after exposure to three concentration levels of Zinc (as Zinc chloride) for 7 days (7 animals were used per test).

	RBC	Hb	HC
Control			
Mean ± S.E.	2.50 ± 0.20	8.51 ± 0.13	23.0 ± 0.50
Range	2.0 — 2.30	8.0 — 8.90	21.0 — 25.0
Low dose			
Mean ± S.E.	1.90 ± 0.21	5.71 ± 0.16	16.50 ± 0.60
Range	1.10 — 2.40	5.30 — 6.50	15.0 — 19.0
Significance	in significant	P < 0.001	P < 0.002
% of change	- 24 %	- 32 %	- 28 %
Intermediate dose			
Mean ± S.E.	1.50 ± 0.16	4.30 ± 0.27	14.0 ± 0.40
Range	1.10 — 2.10	3.0 — 5.0	13.0 — 16.0
Significance	P < 0.02	P < 0.001	P < 0.001
% of change	- 40 %	- 49 %	- 39 %
High dose			
Mean ± S.E.	1.10 ± 0.03	3.63 ± 0.36	9.50 ± 0.20
Range	1.0 - 1.20	2.90 - 5.0	9.0 - 10.0
Significance	P < 0.01	P < 0.001	P < 0.001
% of change	- 56 %	- 57 %	- 58.6 %

Effect of Copper:

Increased erythrocyte count, haemoglobin level and haematocrit value have been noticed in the blood of Oreochromis niloticus as a result of subjection to copper (as cppper sulphate) at 0.126, 0.252 and 0.378 mg/l levels for 7 days as compared with that of the control fish (Table 19).

Treating O. niloticus with the three doses of copper yeilded significant ($p<0.05$) and ($p<0.001$) changes in the erythrocyte counts respectively the increases reached 26%, 26.8% and 28% of the control value in case of the three copper doses treated fish in the otherorder mentioned.

The mean Hb level was 9.18±0.07, 9.81±0.07 and 10.33±0.08 g/100 ml of blood after subjecting O. niloticus to the three doses of copper respectively. This increase was statistically significant ($p<0.01$) in case of the first dose and highly significant ($p<0.001$) in case of the intermediate and high doses respectively. The percentage of increase was 7.8%, 15% and 21% respectively.

The average HC value of the control fish group was 23.0±0.50. This value increased with increasing copper dose to reach 23.80±0.50, 24.0±0.40 and 26.50±0.60 for the low, intermediate and high doses treated fish respectively. However, the increase was insignificant in case of the low and intermediate doses while, it was significant ($p<0.01$) in case of the third high dose where it reached 3.4%, 4.3% and 14% respectively.

Table (19): Mean and range values of red blood cell count (RBC) ($x10^6$), haemoglobin level (Hb) (g/100 ml) and haematocrit value (HC%) of Oreochromis niloticus after subjection to three concentration levels of copper (as copper sulphate) for 7 days (7 animals were used per test).

	RBC	Hb	HC
Control			
Mean ± S.E.	2.50 ± 0.20	8.51 ± 0.13	23.0 ± 0.50
Range	2.0 — 3.20	8.0 — 8.90	21.0 — 25.0
Low dose			
Mean ± S.E.	3.15 ± 0.03	9.18 ± 0.07	23.8 ± 0.50
Range	3.0 — 3.30	9.0 — 9.5	22.0 — 26.0
Significance	P < 0.05	P < 0.01	in significant
% of change	+ 26 %	+ 7.8 %	+ 3.4 %
Intermediate dose			
Mean ± S.E.	3.17 ± 0.02	9.81 ± 0.07	24.0 ± 0.40
Range	3.10 — 3.30	9.50 — 10.0	22.0 — 25.0
Significance	P < 0.05	P < 0.001	in significant
% of change	+ 26.8	+ 15 %	+ 4.3 %
High dose			
Mean ± S.E.	3.20 ± 0.05	10.33 ± 0.08	26.50 ± 0.60
Range	3.0 - 3.40	10.0 - 10.60	25.0 - 28.0
Significance	P < 0.001	P < 0.001	P < 0.01
% of change	+ 28 %	+ 21 %	+ 14 %

It is clear from table (20) that the calculated mean corpuscular volume (MCV)of the fish; oreochromis niloticus raised for 7 days in any of the four single metals (iron, manganes, zinc and copper) showed a general decrease of MCV at most of the three doses used for each metal. The intermediate dose of zinc, however, induced a slight increase of MCV. The four metal treatments resulted in a general decrease of mean corpuscular haemoglobin (MCH) also. However, while manganese and copper produced an increase of the mean corpuscular haemoglobin concentration, (MCH), zinc treatment resulted in a decrease. Iron treatment on the other hand, did not change MCHC appreciably (Table 20).

Table (20): Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) of Oreochromis niloticus treated with single metal (iron, manganese, zinc and copper) at the three dose levels for one week period.

	MCV μm_3	MCH Pg	MCHC %
Control			
Fe – low dose	66.9	24.33	37.02
Fe – Intermediate dose	65.9	23.86	36.2
Fe – High dose			
Mn – Low dose	77.8	30.5	39.0
Mn – Intermediate dose	84.4	33.65	39.8
Mn – High dose	66.6	28.2	42.4
Zn – Low dose	86.8	30.05	34.6
Zn – Intermediate dose	93.3	28.66	30.7
Zn – High dose	86.36	33.0	38.2
Cu – Low dose	75.5	29.1	38.5
Cu – Intermediate dose	75.7	30.9	40.88
Cu – High dose	82.8	32.28	38.98

0.09g/100ml respectively. It is clear that the total serum protein of O. niloticus treated with this dose (intermediate dose) of the mixtures of the metals was highly significantly increased and this increase reached 27%, 64.5%, 102%, 56%,43% and 49% of the mean control value respectively.

Furthermore, the high level of the metal mixtures applied to the fish for one week period caused a more pronouncing increase of the total serum protein. The values were 4.91±0.04, 6.06±0.07, 5.90±0.06, 5.76± 0.09 and 5.98±0.07g/ 100ml for the mixtures of iron and manganese, iron and zinc, manganese and zinc, manganese and copper and zinc and copper respectively, (the mixture of iron and copper was lethal to the fish). Statistical analysis showed that the increase of the mean value of the total serum protein was highly significant ($p<0.001$) when compared with that of the control group. This increase reached 40%, 73%, 68.5%, 62.8% and 70.8% respectively (Table 21).

It is obvious from these data that combination of the four metals with each other at the three doses used resulted in a marked increase in the mean value of the total serum protein of the Nile fish; Oreochromis niloticus. This increase was dose dependent for each metal mixture. The most affecting one was that of iron and copper, while the least affecting metal mixture was iron and manganese at the three dose levels.

Table (21): Mean levels of the total serum protein (g/100ml) of Oreochromis niloticus after exposure to three concentration levels of mixtures of iron and manganese, iron and zinc, iron and copper, manganese and zinc, manganese and copper and zinc and copper for 7 days (7 animals were used per test).

	Control	Fe and Mn	Fe and Zn	Fe and Cu	Mn and Zn	Mn and Cu	Zn and Cu
Low dose							
Mean ± S.E.	3.50±0.1	4.03±0.05	5.30±0.06	6.30±0.05	5.03±0.05	4.40±0.06	4.55±0.09
Range	3.1—4.0	3.8—4.2	5.1—5.3	6.1—6.5	4.8—5.2	4.2—4.6	4.2—4.9
Significance		P<0.01	P<0.001	P<0.001	P<0.001	P<0.002	P<0.002
% of change		+15%	+51%	+80%	+43.7%	+25.7%	+30%
Intermediate dose							
Mean ± S.E.		4.45±0.05	5.76±0.06	7.10±0.07	5.46±0.05	5.01±0.06	5.23±0.09
Range		4.2—4.6	5.5—5.9	6.8—7.4	5.3—5.7	4.8—5.3	4.9—5.5
Significance		P<0.002	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
% of change		+27%	+64.5%	+102%	+56%	+43%	+49%
High dose							
Mean ± S.E.		4.91±0.04	6.06±0.07	All fish	5.90±0.06	5.76±0.09	5.98±0.07
Range		4.8—5.1	5.9—6.3	expired	5.7—6.1	5.5—6.1	5.7—6.3
Significance		P<0.001	P<0.001		P<0.001	P<0.001	P<0.001
% of change		+40%	+73%		+68.5%	+62.8%	+70.8%

Table (22): Mean levels of glutamate oxaloacetate transaminase (GOT) (IU/min/l) activity of the serum of Oreochromis niloticus after subjection to three concentration levels of mixtures of iron and manganese, iron and zinc, iron and copper, manganese and zinc, manganese and copper and zinc and copper for 7 days (7 animals were used per test).

	Control	Fe and Mn	Fe and Zn	Fe and Cu	Mn and Zn	Mn and Cu	Zn and Cu
Low dose							
Mean ± S.E.	27.0±0.7	29.0±0.7	28.5±0.9	36.83±0.7	30.33±0.5	27.66±0.5	29.66±0.3
Range	24.0—29.0	26.0—32.0	26.0—33.0	35.0—40.0	29.0—33.0	26.0—30.0	29.0—31.0
Significance		insignificant	insignificant	P<0.001	P<0.02	insignificant	P<0.05
% of change		+7.4%	+5.5%	+36%	+12%	+2.4%	+9.8%
Intermediate dose							
Mean ± S.E.		32.16±0.4	29.83±0.5	40.16±0.7	31.50±0.5	30.50±0.2	32.50±0.3
Range		31.0—34.0	28.0—32.0	37.0—41.0	30.0—33.0	30.0—31.0	31.0—34.0
Significance		P<0.01	P<0.05	P<0.001	P<0.01	P<0.01	P<0.002
% of change		+19%	+10%	+48.7	+16.6%	+12.9%	+20%
High dose							
Mean ± S.E.		33.66±0.8	33.16±0.4	All fish	34.33±0.4	33.66±0.4	34.8±0.5
Range		30.0—36.0	32.0—35.0	expired	33.0—36.0	32.0—35.0	33.0—37.0
Significance		P<0.01	P<0.002		P<0.001	P<0.002	P<0.001
% of change		+24.6%	+22.8%		+27%	+24.6%	+28.8%

Glutamate pyruvate transaminase activity (GPT) :

The data presented in table (23) depict the mean activity of GPT (IU/min/l) enzyme of the serum of Oreochromis niloticus after exposing the fish to the three doses of mixtures of iron and manganese, iron and zinc, iron and copper, manganese and zinc, manganese and copper and zinc and copper for 7 days as compared with that of the control group. It is evident that the GPT activity of the serum of O. niloticus showed a steady increase with the increase of the dose mixtures.

The mean value of the control group was 9.0±0.4 IU/min/l whereas that of those fish groups raised in low dose mixtures was 10.16±0.2, 10.50±0.3, 14.20±0.1, 13.80±0.1, 14.80±0.2 and 12.50±0.2 IU/min/l respectively. Statistical analysis showed that the increase of the mean serum GPT activity was significant for all of the forementioned mixtures as compared with that of the control group. Such increases were marked in case of manganese and copper (64%), iron and copper (57.7%) and manganese and zinc (53%) treated fish groups,moderate in case of zinc and copper (38.8%) treated group and slight in case of iron and zinc (16.6%) and iron and manganese (12.8%) treated groups respectively.

The mean GPT activity of the serum of O. niloticus exposed to the intermediate dose of the previously cited metal mixtures was 10.33±0.1, 12.0±0.3, 15.50±0.2, 17.0±0.3 17.60±0.8 and 13.60±0.1 IU/min/l respectively. It is clear that the data declare a pronouncing increase of the mean serum GPT activity which was highly significant ($p<0.001$) for

most of the metal mixtures used as compared with that of the control group. The increase reached 14.7%, 33%, 72%, 88.8% 95.5% and 51% for the abovementioned metal mixtures treated groups respectively.

The high dose of the metal mixtures; iron and manganese, iron and zinc, iron and copper, manganese and zinc, manganese and copper and zinc and copper also resulted in marked increases of mean serum GPT activity. The mean values were 12.16±0.2, 13.50±0.2, 18.50±0.2, 18.0± 0.4 and 15.60±0.4 IU/min/l compared with 9.0±0.4 IU/min/l for the control group. The increases of serum GPT mean activity were highly significant ($p<0.001$) and reached 35%, 50%, 105%, 100% and 73% of the mean control value respectively.

As it can be seen from the previously recorded observations that serum GPT activity was much affected by the combined metal treatment than serum GOT which might be an indication of liver function disturbances. It is clear also that iron and copper high dose mixture was lethal on the fish. However, least effect of combined metal treatment was noticed in case of iron and manganese treated fish groups. The marked effects were recorded in case of manganese and zinc and manganese and copper treated groups respectively.

Table (23): Mean levels of glutamate pyruvate transaminase (GPT) (IU/min/l) activity of the serum of Oreochromis niloticus after exposure to three concentration levels of mixtures of iron and manganese, iron and zinc, iron and copper, manganeseand zinc, manganese and copper and zinc and copper for 7 daysaz~ and day (7 animals were used per test).

	Control	Fe and Mn	Fe and Zn	Fe and Cu	Mn and Zn	Mn and Cu	Zn and Cu
Low dose							
Mean ± S.E.	9.0±0.4	10.16±0.2	10.50±0.3	14.20±0.1	13.80±0.1	14.80±0.2	12.50±0.2
Range	7.0—10.0	9.0—11.0	9.0—12.0	14.0—15.0	13.0—14.0	14.0—16.0	12.0—13.0
Significance		P<0.05	P<0.05	P<0.001	P<0.001	P<0.001	P<0.002
% of change		+12.8	+16.6%	+57.7%	+53%	+64%	+38.8
Intermediate dose							
Mean ± S.E.		10.33±0.1	12.0±0.3	15.50±0.2	17.0±0.3	17.60±0.8	13.60±0.1
Range		10.0—11.0	11.0—13.0	15.0—16.0	16.0—18.0	15.0—20.0	13.0—14.0
Significance		P<0.05	P<0.01	P<0.001	P<0.001	P<0.001	P<0.001
% of change		+14.7%	+33%	+72%	+88.8%	+95.5%	+51%
High dose							
Mean ± S.E.		12.16±0.2	13.50±0.2	All fish	18.50±0.2	18.0±0.4	15.6±0.4
Range		11.0—13.0	13.0—14.0	expired	18.0—19.0	16.0—19.0	14.0—17.0
Significance		P<0.002	P<0.001		P<0.001	P<0.001	P<0.001
% of change		+35%	+50%		+105%	+100%	+73%

Acid phosphatase activity (AP) :

Table (24) summerizes the data of serum AP activity (kind and king Units/l) of the serum of Oreochromis niloticus subjected to three doses of mixtures of iron and manganese, iron and zinc, iron and copper, manganese and zinc, manganese and copper and zinc and copper for one week. It is clear that the mean AP activity of the serum of O. niloticus was increased as the dose of the combined metals was increased.

The mean serum AP activity of the control fish was 2.80±0.07 kind and king Units/l. This value was increased to 3.20±0.07, 3.40±0.11, 4.40±0.07, 3.50±0.09, 3.30±0.05 and 3.50±0.15 Kind and king Units/l after subjecting the fish groups to the low dose of paired metals cited above respectively. The data demonstrate that the enzyme activity was increased significantly for all of the paired metal treated groups with this dose level. With exception of iron and copper mixture treated group of fish which showed marked increase; (57%). The increase of serum AP activity was of a moderate level (14%, 21%25%,17.8% and 25%) respectively.

The fish groups treated with the intermediate combined metals (iron and manganese, iron and zinc, iron and copper, manganese and zinc, manganese and copper and zinc and copper) showed more increases of the serum enzyme activity. The mean value of the enzyme activity was 3.50±0.15, 3.50±0.03, 5.50±0.11, 3.70±0.05, 3.60±0.12 and 4.30+0.05 kind and king Units/l for the control fish group and the above mentioned groups respectively. Statistically this increase was generally highly

Table (24): Mean levels of acid phosphatase (AP) activity (kind and king Units/l) of the serum of Oreochromis niloticus after exposure to three concentration levels of mixtures of iron and manganese, iron and zinc, iron and copper, manganese and zinc, manganese and copper and zinc and copper for 7 days (7 animals were used per test).

	Control	Fe and Mn	Fe and Zn	Fe and Cu	Mn and Zn	Mn and Cu	Zn and Cu
Low dose							
Mean ± S.E.	2.80±0.07	3.20±0.07	3.4±0.11	4.40±0.07	3.5±0.09	3.30±0.05	3.50±0.15
Range	2.50—3.0	2.9—3.3	3.0—3.9	4.1—4.6	2.2—4.0	3.2—3.5	3.0—3.9
Significance		P<0.02	P<0.01	P<0.001	P<0.01	P<0.01	P<0.02
% of change		+14%	+21%	+57%	+25%	+17.8%	+25%
Intermediate dose							
Mean ± S.E.		3.50±0.15	3.50±0.03	5.5±0.11	3.70±0.05	3.60±0.12	4.3±0.05
Range		3.0—4.0	3.5—3.7	5.0—5.9	3.6—4.0	3.0—3.9	4.2—4.5
Significance		P<0.02	P<0.001	P<0.001	P<0.001	P<0.01	P<0.001
% of change		+25%	+25%	+96%	+32%	+28.5%	+53.5%
High dose							
Mean ± S.E.		4.10±0.03	4.00±0.07	All fish	4.20±0.06	3.80±0.01	4.80±0.03
Range		4.0—4.3	3.9—4.4	expired	4.0—4.4	3.7—3.8	4.7—4.9
Significance		P<0.001	P<0.001		P<0.001	P<0.001	P<0.001
% of change		+46%	+42.8%		+50%	+35.7%	+71%

Alkaline phosphatase activity (AlP) :

The mean level of serum AlP (kind and king Units/l) activity of the Nile fish; Oreochromis niloticus after exposure to three dose mixtures of iron and manganese, iron and zinc, iron and copper, manganse and zinc, manganese and copper and zinc and copper for 7 days is shown in table (25). It is clear from the table that the mean serum AlP activity of the control fish was 21.0±0.4 kind and king Units/l; while that of the fish groups exposed to the low dose of the paired metals was 23.80±0.3, 31.30±0.7, 32.60±0.5, 29.50±0.5, 29.00±0.4 and 24.60±0.5 kind and king Units/l respectively. The data revealed an increase of AlP activity which was statistically significant for all metal mixture treated groups. The increase reached 13%, 49%, 55%, 40%, 38% and 17% respectively.

Treating the fish with the intermediate metal mixture doses resulted in a more increase of the serum enzyme activity. The mean activity reached to 27.20±0.6, 32.20±0.3, 35.20±0.6, 31.8±0.2, 32.0±0.4 and 30.20±0.3 kind and king Units/l for the abovementioned combined metal treated groups respectively. Statistically these increases were highly significant ($p<0.001$). They reached 29.5%, 53%, 67%, 51%, 52% and 43.8% respectively.

It is obvious that there was a pronouncing increase of the mean level of serum AlP activity of the high dose paired metals; iron and mangaese, iron and zinc, manganese and zinc, manganese and copper and zinc and copper treated groups when compared with the mean level of the control fish. The mean levels were 30.60±0.5, 33.30±0.7, 34.50±0.3, 34.80±0.4

and 32.20±0.3 kind and king Units/l respectively as compared with 21.0± 0.4 kind and king Units/l for the control group. The increases were statistically highly significant ($p<0.001$) and reached 45.7%, 58.5%, 64%, 65.7% and 53% of that of the mean control fish group respectively.

It is obvious that there was an increase in the mean level of serum AIP activity of all of the fish groups treated with any of the obovementioned metal mixture doses. The intermediate iron and copper dose produced the highest serum enzyme activity (at the high iron and copper mixture dose fishes were expired within few hours from the starting of the experiment). Iron and manganese metal mixture resulted in the least effect at the three doses used.

Table (25): Mean levels of alkaline phosphatase (AlP) activity (kind and king Units/l) of the serum of Oreochromis niloticus after exposure to three concentration levels of mixtures of iron and manganese, iron and zinc, iron and copper, manganese and zinc, manganese and copper and zinc and copper for 7 days (7 animals were used per test).

	Control	Fe and Mn	Fe and Zn	Fe and Cu	Mn and Zn	Mn and Cu	Zn and Cu
Low dose							
Mean ± S.E.	21.0±0.4	23.80±0.3	31.30±0.7	32.60±0.5	29.50±0.5	29.0±0.4	24.60±0.5
Range	20.0—22.0	22.0—24.0	29.0—31.0	31.0—35.0	28.0—31.0	28.0—30.0	23.0—26.0
Significance		P<0.01	P<0.001	P<0.001	P<0.001	P<0.001	P<0.01
% of change		+13%	+49%	+55%	+40%	+38%	+17%
Intermediate dose							
Mean ± S.E.		27.20±0.6	32.20±0.3	35.2±0.6	31.80±0.2	32.0±0.4	30.20±0.3
Range		27.0—29.0	31.0—33.0	33.0—38.0	31.0—33.0	30.0—33.0	29.0—31.0
Significance		P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
% of change		+29.5%	+53%	+67%	+51%	+52%	+43.8%
High dose							
Mean ± S.E.		30.60±0.5	33.30±0.7	All fish	34.50±0.3	34.80±0.4	32.20±0.3
Range		29.0—31.0	31.0—35.0	expired	34.0—36.0	33.0—36.0	31.0—33.0
Significance		P<0.001	P<0.001		P<0.001	P<0.001	P<0.001
% of change		+45.7%	+58.5%		+64%	+65.7%	+53%

Effect of combined metals on total liver protein:

The results given in table (26) indicate that the mean value of the total liver protein increased as the concentration level of the paired metals increased. The average value of the control group was 0.018± 0.003 g/g liver tissue. This value increased to 0.022±0.0008, 0.029± 0.001, 0.033±0.001, 0.031±0.0007, 0.027±0.0008, 0.028±0.001 g/g liver as a result of subjeting the fish to the low concentration levels of mixtures of iron and manganese, iron and zinc, iron and copper, manganese and zinc, manganese and copper and zinc and copper respectively. Statistically the increase was significant except for iron and manganese mixture group. The percentage reached 22%, 61%, 83%, 72%, 50% and 55.5% for the previously mentioned mixtures of metals treated fish respectively.

The intermediate dose of the paired metals also signifiantly increased the average value of the total liver protein (Table 26). The increases were 55.5%, 77.7%, 111%, 100%, 100% and 94% due to subjecting the fish groups to iron and manganese, iron and zinc, iron and copper, manganese and zinc, manganese and copper and zinc and copper mixtures respectively.

Similarly total liver protein of fish groups treated with the same metal mixtures but at a high dose markedly increased after 7days of treatment. The increase amounted to 100%, 88.8%, 122%, 138% and 133% for the forementioned mixtures respectively. It is worthy to mention that these increases were statistically signifiant for all of the

above mentioned mixtures of metals. However, the high mixture dose of iron and copper was a lethal to the fish and they died within few hours from the starting of the experiment.

It is obvious that combination of the four metals had affected markedly the average value of the total liver protein. The increase was deeper with increased doses of the paired metals. The high iron and copper mixture dose was a lethal one.

Table (26): Mean levels of the total liver protein (g/g tissue) of Oreochromis niloticus after subjection to three levels of mixtures of iron and manganese, iron and zinc, iron and copper, manganese and zinc, manganese and copper and zinc and copper (as salts) for 7 days (7 animals were used per test).

	Control	Fe and Mn	Fe and Zn	Fe and Cu	Mn and Zn	Mn and Cu	Zn and Cu
Low dose							
Mean ± S.E.	0.018±0.003	0.022±0.0008	0.029±0.001	0.033±0.001	0.031±0.0007	0.027±0.0008	0.028±0.001
Range	0.011—0.031	0.020—0.025	0.027—0.035	0.029—0.038	0.028—0.034	0.025—0.030	0.022—0.036
Significance		insignificant	P<0.05	P<0.01	P<0.02	P<0.05	P<0.05
% of change		+22%	+61%	+83%	+72%	+50%	+55.5%
Intermediate dose							
Mean ± S.E.		0.028±0.0008	0.032±0.009	0.038±0.001	0.036±0.003	0.036±0.008	0.035±0.001
Range		0.025—0.030	0.030—0.36	0.032—0.042	0.022—0.051	0.034—0.039	0.028—0.041
Significance		P<0.05	P<0.02	P<0.01	P<0.02	P<0.01	P<0.01
% of change		+55.5%	+77.7%	+111%	+100%	+100%	+94%
High dose							
Mean ± S.E.		0.036±0.001	0.034±0.001	All fish	0.040±0.004	0.043±0.008	0.042±0.001
Range		0.032—0.041	0.027—0.041	expired	0.027—0.053	0.041—0.046	0.036—0.046
Significance		P<0.01	P<0.01		P<0.01	P<0.002	P<0.002
% of change		+100%	+88.8%		+122%	+138%	+133%

Effect of Combined Metals on Liver Enzyme Activity

Glutamate oxaloacetate transaminase (GOT) activity:

The data of the liver GOT activity of the control group and after subjecting the fish to three concentration levels (low, intermediate and high doses) of paired metals are shown in table (27). It is obvious that the mean level of GOT activity of the control group was 2.613±0.07 IU/g tissue/min. This value decreased to reach 1.236±0.05, 1.282±0.06, 0.570±0.05, 1.345±0.07, 1.240±0.06, 1.310±0.04 IU/g tissue/min after treating the fish groups to the low dose of the mixtures of iron and manganese, iron and zinc, iron and copper, manganese and zinc, manganese and copper and zinc and copper respectively. This decrease was statistically significant ($P<0.001$) for all of the above mentioned paired metals fish treated groups. It amounted to 52.6%, 50.9%, 78%, 48.5%, 52.5% and 49.8% of the mean control level respectively.

Applying the intermediate dose of the paired metals to O. niloticus resulted in a more deeper decrease of liver GOT activity (Table 27). The decrease was statistically significant where it reached 77%, 61%, 83%, 70.9%, 69.5% and 80.5% of the mean value of the control group respectively.

With the exception of fish group treated with iron and copper mixture, high concentration level of the combined metals treatment resulted also in a more decrease of the mean liver GOT activity. The mean value reached 0.298±0.01, 0.794±0.02, 0590±0.02, 0.626±0.02 and 0.374±0.05 respectively. The data revealed that the derease of the mean

liver GOT activity was statistically highly significant (P< 0.001) for all of the above mentioned paired metals treated fish groups as compared with that of the control group. The percentage of decrease reached 88.5%, 69.5%, 77%, 76%, and 85.7% respectively. It is clear that the high dose of iron and copper mixture was lethal to the fish. Fish exposed to that mixture dose died within few hours of subjection to that metal salt dose.

It is evident from table (27) that there was a gradual decrease of the mean level of liver GOT activity as a result of increasing the dose of the mixture of the metals. The results reflect the increased serum GOT activity due to paired metal application. Iron and copper high dose mixture was a lethal dose to the fish.

Table (27): Mean values of glutamate oxaloacetate transaminase (GOT) (IU/g tissue/min) activity of the liver of Oreochromis niloticus after exposure to three levels of mixtures of iron and manganese, iron and zinc, iron and copper, manganese and zinc, manganese and copper and zinc and copper for 7 days (7 animals were used per test).

	Control	Fe and Mn	Fe and Zn	Fe and Cu	Mn and Zn	Mn and Cu	Zn and Cu
Low dose							
Mean ± S.E.	2.613±0.07	1.236±0.05	1.282±0.06	0.570±0.05	1.345±0.07	1.240±0.06	1.310±0.04
Range	2.459—2.989	1.020—1.407	1.066—1.500	0.341—0.751	1.129—1.590	1.030—1.441	1.194—1.308
Significance		P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
% of change		- 52.6%	-50.9%	-78%	-48.5%	-52.5%	-49.8%
Intermediate dose							
Mean ± S.E.		0.589±0.02	1.007±0.03	0.442±0.03	0.760±0.02	0.796±0.02	0.509±0.02
Range		0.497—0.656	0.917—1.150	0.322—0590	0.695—0.857	0.723—0.934	0.452—0.593
Significance		P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
% of change		-77%	-61%	-83%	-70.9%	-69.5%	-80.5%
High dose							
Mean ± S.E.		0.298±0.01	0.794±0.02	All fish	0.590±0.02	0.626±0.02	0.374±0.05
Range		0.246—0.351	0.737—0.913	expired	0.529—0.686	0.553—0.686	0.219—0.588
Significance		P<0.001	P<0.001		P<0.001	P<0.001	P<0.001
% of change		-88.5%	-69.9%		-77%	-76%	-7%

Glutamate Pyruvate transaminase (GPT) activity:

The data presented in table (28) show the mean level of liver GPT activity (IU/g tissue/min) after subjecting Oreochromis niloticus to three concentration levels of mixtures of iron and manganese, iron and zinc, iron and copper, manganese and zinc, manganese and copper and zinc and copper (as salts). The main effect of combined metal application on liver GPT activity was a gradual and dose dependent inhibition.

The mean level of liver GPT activity of the control fish was 0.653± 0.04 IU/g tissue/min whereas that for fishes raised in the low dose of the mixtures of iron and manganese, iron and zinc, iron and copper, manganese and zinc, manganese and copper and zinc and copper were 0.510±0.02, 0.391±0.02, 0.348±0.06, 0.532±0.01, 0.502±0.02 and 0.413±0.02 IU/g tissue/min respectively. Statistically the decrease of GPT mean activity was significant for all of low dose paired metal treated fish groups. The decrease reached 21.8%, 40%, 46.7%, 18.5%, 23% and 36.7% of the value recorded for the control group respectively.

The inhibition of liver GPT activity was progressively noticed as a result of treatment with the intermediate dose of the paired mixtures of metals (Table 28). The mean activity of the enzyme was 0.653±0.04 IU/g tissue/min in case of the control group which decreased to 0.467±0.01, 0.281±0.01, 0.222±0.02, 0.310±0.01, 0.159±0.008 and 0.385±0.01 IU/g tissue/min after exposing the fish to iron and manganese, iron and zinc, iron and copper, manganese and zinc, manganese and copper and zinc and copper mixtures respectively. This decrease of mean liver GPT

activity was statistically highly significant (P<0.001) for all of the above mentioned mixtures. The data depict a decrease of 28%, 56.9%, 66%, 52.5%, 75.6% and 41% of the control group respectively.

Liver mean GPT activity was pronouncly decreased after subjecting O. niloticus groups to the high dose of the mixtures of the metals. Thus the activity decreased to reach 0.140±0.006, 0.152±0.008, 0.181±0.01, 0.149±0.008 and 0.156±0.009 IU/g tissue/min after rearing the fish groups in water containing iron and manganese, iron and zinc, manganese and zinc, manganese and copper and zinc and copper high dose mixtures respectively. This decrease was statistically highly significant (P<0.001) for all of the above mentioned pairs of metals. It amounted to 78.5%, 76.7%, 72%, 77% and 76% of the value recorded for the control respectively. Fish subjected to the high dose of iron and copper mixture died within few hours.

It can be seen from the previously cited data that there was a more deeper decrease in the mean liver GPT activity with increased dose of the paired metals.

activity. The mean value of the enzyme of the control group was 0.452± 0.007 kind and king units/g tissue while that of the abovementioned mixtures was 0.159±0.01, 0.146±0.01, 0.152±0.004, 0.141±0.008 and 0.054±0.008 kind and king units/g tissue respectively. The decreases were statistically highly significant ($P<0.001$) with percentage of 67.6%, 64.8%, 66%, 68.8% and 88% of the control value respectively.

It is clear that applying the mixtures of the four metals at different concentration levels for one week period resulted in a marked decrease of mean liver AP activity and this decrease was more pronouncing when zinc and copper mixture was used at any of the doses recorded. Also the intermediate dose of iron and copper showed the same inhibitory effect of zinc and copper mixture on liver AP activity.

Table (29): Mean values of acid phosphatase (AP) (kind and king Units/g tissue) activity of the liver of Oreochromis niloticus after exposure to three levels of mixtures of iron and manganese, iron and zinc, iron and copper, manganese and zinc, manganese and copper and zinc and copper for 7 days (7 animals were used per test).

	Control	Fe and Mn	Fe and Zn	Fe and Cu	Mn and Zn	Mn and Cu	Zn and Cu
Low dose							
Mean ± S.E.	0.452±0.007	0.379±0.005	0.270±0.008	0.218±0.01	0.335±0.005	0.310±0.007	0.172±0.005
Range	0.429—0.483	0.355—0.398	0.228—0.291	0.179—0.239	0.322—0.361	0.218—0.393	0.156—0.195
Significance		P<0.002	P<0.001	P<0.001	P<0.001	P<0.01	P<0.001
% of change		-16%	-40%	-51.7%	-25.8%	-31%	-61.9%
Intermediate dose							
Mean ± S.E.		0.278±0.02	0.232±0.01	0.081±0.005	0.270±0.002	0.193±0.007	0.081±0.005
Range		0.203—0.344	0.178—0.273	0.066—0.099	0.257—0.276	0.161—0.214	0.068—0.098
Significance		P<0.002	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
% of change		-38%	-48.6%	-82%	-40%	-57%	-82%
High dose							
Mean ± S.E.		0.159±0.01	0.146±0.01	All fish	0.152±0.004	0.141±0.008	0.054±0.008
Range		0.118—0.201	0.118—0.201	expired	0.132—0.167	0.116—0.186	0.022—0.080
Significance		P<0.001	P<0.001		P<0.001	P<0.001	P<0.001
% of change		-64.8%	-67.6%		-66%	-68.8%	-88%

Alkaline phosphatase (AlP) activity:

The mean level of alkaline phosphatase activity of the liver of Oreochromis niloticus after exposure for one week period to three doses of mixtures of iron and manganese, iron and zinc, iron and copper, manganese and zinc, manganese and copper and zinc and copper is shown in table (30). It is clear that the mean level of the control group was 2.154+0.02 kind and king units/g tissue while that for those fishes subjeted to the low dose of the forementioned mixtures of metals was 1.772±0.04, 1.539±0.07, 0.861±0.02, 1.234±0.08, 1.592±0.06 and 2.359±0.06 kind and king units/g tissue respectively. Statistical analysis showed that there was a general decrease of the mean liver AlP activity and this decrease was significant for most of the mixtures as compared with that of the control group. The decreases were 17.7%, 28.5%, 60%, 42.7% and 26% respectively. However, liver AlP activity of the fish group treated with the low dose of zinc and copper mixture increased slightly (9.5%) but significantly ($P<0.05$).

Treating the fish groups with the intermediate dose of metal mixtures (as salts) for 7days resulted in a deeper decrease of liver AlP activity. The mean values of the enzyme activity were 1.403±0.05, 1.473±0.08, 0.535±0.01, 0.851±0.01, 1.574±0.07 and 1.779±0.08 for the mixtures of iron and manganese, iron and zinc, iron and copper, manganese and zinc, manganese and copper and zinc and copper respectively. The decrease was proved to be statistically significant for all of the above mentioned mixtures of metals as compared with that of the control group. The percentage of decrease reached 34.8%, 31.6%, 75%, 60%, 26.9% and 17% respectively.

Furthermore, the high metal mixture dose showed a more deeper derease of the enzyme activity (Table 30). Statistical analysis showed that the derease was generally highly significant and reached 69.9%, 37%, 69.6%, 37.7% and 37.8% for iron and manganese, iron and zinc, manganese and zinc, manganese and copper and zinc and copper mixtures treated fish groups respectively.

It is clear from the forementioned data that with exception of zinc and copper low mixture dose all of paired metals decreased liver AlP activity and that this decrease was dose dependent. However,the most marked inhibition of the enzyme activity was found in case of iron and copper intermediate dose treated fish group. The least affected liver AlP activity was that of the fish group treated with manganese and copper. Furthermore, that of fish treated with the low dose of zinc and copper mixture was slightly but statistically significantly increased.

Table (30): Mean values of alkaline phosphatase (AlP) (kind and king Units/g tissue) activity of the liver of Oreochromis niloticus after exposure to three levels of mixtures of iron and manganese, iron and zinc, iron and copper, manganese and zinc, manganese and copper and zinc and copper for 7 days (7 animals were used per test).

	Control	Fe and Mn	Fe and Zn	Fe and Cu	Mn and Zn	Mn and Cu	Zn and Cu
Low dose							
Mean ± S.E.	2.154±0.02	1.772±0.04	1.539±0.07	0.861±0.02	1.234±0.08	1.592±0.06	2.359±0.06
Range	2.065—2.197	1.641—1.938	1.300—1.834	0.765—0.75	1.005—1.551	1.402—1.775	2.056—2.537
Significance		P<0.002	P<0.002	P<0.001	P<0.001	P<0.001	P<0.05
% of change		-17.7%	-28.5%	-60%	-42.7%	-26%	+9.5%
Intermediate dose							
Mean ± S.E.		1.403±0.05	1.473±0.08	0.535±0.01	0.851±0.01	1.574±0.07	1.779±0.08
Range		1.194—1.666	1.100—1.735	0.502—0.596	0.802—0.893	1.29—1.888	1.428—1.990
Significance		P<0.001	P<0.002	P<0.001	P<0.001	P<0.002	P<0.51
% of change		-34.8%	-31.6%	-75%	-60%	-26.9%	-17%
High dose							
Mean ± S.E.		0.647±0.01	1.352±0.05	All fish	0.654±0.02	1.340±0.03	1.338±0.06
Range		0.792—0.900	1.095—1.520	expired	0.591—0.702	1.260—1.515	1.071—1.555
Significance		P<0.001	P<0.002		P<0.001	P<0.001	P<0.001
% of change		-69.9%	-37%		-69.6%	-37.7%	-37.8%

Effect of paired metals on total blood protein and the blood serum enzymes

In this set of experiments fish groups were reared in paired metal levels including iron and manganese iron and zinc, iron and copper, manganese and zinc, manganese and copper and zinc and copper at the same previously mentioned doses (low, intermediate and high doses) for a one week period after which analysis was carried out.

Effect of metal Combination on total serum protein :

Applying any of the three doses of paired metal mixtures (iron and manganese, iron and zinc, iron and copper, manganese and zinc, manganese and copper and zinc and copper) on the Nile fish; Oreochromis niloticus for a period of one week resulted in an increase of total serum protein of the fish (Table 21). The table shows that the mean blood serum protein value of the control fish group was 3.50±0.10g/ 100ml while that of the forementioned low dose combined metal fish treated groups was 4.03±0.05,5.30±0.06,6.30±0.05,5.03±0.05,4.40±0.06 and 4.55±0.09g/100ml respectively. The increase was statistically highly significant for all of the abovementioned metal mixture treated groups. It reached 15%, 51%, 80%, 43.7%, 25.7% and 30% of the mean control value respectively.

Similarly the total serum protein increased markedly after raising the fish in water containing the intermediate dose of the metal mixtures for a one week period. The mean total serum protein value increased to reach 4.45±0.05, 5.76±0.06, 7.10±0.07, 5.46±0.05, 5.01±0.06 and 5.23±

0.09g/100ml respectively. It is clear that the total serum protein of O. niloticus treated with this dose (intermediate dose) of the mixtures of the metals was highly significantly increased and this increase reached 27%, 64.5%, 102%, 56%,43% and 49% of the mean control value respectively.

Furthermore, the high level of the metal mixtures applied to the fish for one week period caused a more pronouncing increase of the total serum protein. The values were 4.91±0.04, 6.06±0.07, 5.90±0.06, 5.76± 0.09 and 5.98±0.07g/ 100ml for the mixtures of iron and manganese, iron and zinc, manganese and zinc, manganese and copper and zinc and copper respectively, (the mixture of iron and copper was lethal to the fish). Statistical analysis showed that the increase of the mean value of the total serum protein was highly significant ($p<0.001$) when compared with that of the control group. This increase reached 40%, 73%, 68.5%, 62.8% and 70.8% respectively (Table 21).

It is obvious from these data that combination of the four metals with each other at the three doses used resulted in a marked increase in the mean value of the total serum protein of the Nile fish; Oreochromis niloticus. This increase was dose dependent for each metal mixture. The most affecting one was that of iron and copper, while the least affecting metal mixture was iron and manganese at the three dose levels.

Table (31): Mean values of the total brain protein (g/g tissue) of Oreochromis niloticus after subjection to three levels of mixtures of iron and manganese, iron and zinc, iron and copper, manganese and zinc, manganese and copper and zinc and copper for 7 days (7 animals were used per test).

	Control	Fe and Mn	Fe and Zn	Fe and Cu	Mn and Zn	Mn and Cu	Zn and Cu
Low dose							
Mean ± S.E.	0.021±0.0003	0.032±0.004	0.036±0.0008	0.040±0.0005	0.031±0.005	0.033±0.0008	0.032±0.004
Range	0.020—0.023	0.018—0.050	0.033—0.039	0.039—0.041	0.017—0.050	0.030—0.036	0.018—0.050
Significance		P<0.05	P<0.001	P<0.001	insig	P<0.001	P<0.05
% of change		+52%	+71%	+90%	+47.6%	+57%	+52%
Intermediate dose							
Mean ± S.E.		0.037±0.0005	0.039±0.0005	0.045±0.009	0.039±0.008	0.036±0.0003	0.040±0.01
Range		0.035—0.039	0.037—0.040	0.016—0.86P	0.016—0.078	0.035—0.037	0.009—0.092
Significance		P<0.001	P<0.001	P<0.05	insig.	P<0.001	insig.
% of change		+76%	+85.7%	+114%	+85.7%	+71%	+90%
High dose							
Mean ± S.E.		0.042±0.007	0.040±0.0005	All fish	0.047±0.005	0.039±0.0003	0.052±0.007
Range		0.018—0.072	0.038—0.042	expired	0.032—0.067	0.038—0.040	0.018—0.077
Significance		P<0.05	P<0.001		P<0.01	P<0.001	P<0.02
% of change		+100%	+90%		+123.8%	+85.7%	+147%

Glutamate Oxaloactate transaminase (GOT) activity:

The mean and range values of glutamate oxaloacetate transaminase (GOT) (IU/ g tissue/min) activity of the brain of the Nile fish; Oreochromis niloticus after treatment with three concentration levels of mixtures of iron and manganese, iron and zinc, iron and copper, manganese and zinc, manganese and copper and zinc and copper for one week are shown in table (32). It is clear from the table that combination of the metals at different doses resulted in a general decrease of the enzyme activity of the brain of O. niloticus.

The mean level of GOT activity of the control group was 2.540± 0.10 IU/g tissue/min. This value decreased to 1.881±0.01, 0.945±0.01, 0.857±0.01, 1.122±0.02, 2.272±0.07 and 1.546±0.10 IU/g tissue/min after treating the fish with the low dose of the metal mixtures previously cited for one week. Statistical analysis showed that the decrease of brain GOT activity was highly significant for all of the above mentioned mixtures treated fish except for manganese and copper mixture treated group which showed insignificant decrease. The percentages of decrease recorded were 25.9%, 62.7%, 66%, 55.8%, 10.5% and 39% respectively.

The intermediate dose applied to the fish groups treated with iron and manganese, iron and zinc, iron and copper, manganese and zinc, manganese and copper and zinc and copper mixtures showed more pronouncing decreases of brain GOT activity (Table 32). These decreases reached 53%, 65.9%, 78%, 77.5%, 47% and 50.6% respectively.

Glutamate pyravate transaminase (GPT) activity:

Table (33) shows the data of the activity of glutamate pyruvate transaminase (GPT) (IU/g tissue/min) of the brain of Oreochromis niloticus after exposure to three concentration levels of mixtures of iron and manganese, iron and zinc, iron and copper, manganese and zinc, manganese and copper and zinc and copper for 7days as compared with that of the control fish group. It is evident that the mean level of GPT activity of the brain of O. niloticus raised in normal water was 0.985± 0.05 IU/g tissue/min whereas that of those groups treated with the low dose of mixtures of the previously mentioned groups for 7days was 0.590±0.03, 0.653±0.02, 0.720±0.02, 0.390±0.002, 0.740±0.03 and 0.743±0.03 IU/g tissue/min respectively. Statistically the decrease of the mean level of brain GPT activity was significant for all the mixtures of metals. The decrease was 40%, 33%, 26.9%, 60%, 24.8% and 24.5% respectively.

It is clear also that the mean level of brain GPT activity of the fish groups exposed to intermediate dose of the mixtures of the metals showed further decreases. These levels reached 0.440±0.01, 0.449±0.02, 0.305±0.01, 0.195±0.001, 0.446±0.03 and 0.489±0.03 IU/g tissue/min for the mixtures of iron and manganese, iron and zinc, iron and copper,manganese and zinc, manganese and copper and zinc and copper respectively compared with 0.985±0.05 IU/g tissue/min for the control group. The decrease of brain GPT mean level of activity was highly significant ($P<0.001$) for all the forementioned mixtures of metals. These decreases of the mean brain GPT activity reached 55%, 54%, 69%, 80%, 54.7% and 50% respectively.

The data (Table 33) depict also that the high dose metal mixtures induced a highly significant ($P<0.001$) decrease of the mean brain GPT activity. The mean value of the control group was 0.985±0.05 IU/g tissue/min. Those of the treated fish were 0.200±0.01, 0.216±0.001, 0.097±0.0006, 0.184±0.003 and 0.238±0.02 IU/g tissue/min for iron and manganese, iron and zinc, manganese and zinc, manganese and copper and zinc and copper salt mixtures treated groups respectively. These decreases of the mean brain GPT activity reached 79.6%, 78%, 90%, 81% and 75.8% respectively.

It can be seen from the previously cited data that there was a dose dependent decrease of the brain GPT activity of O. niloticus subjected to the combined metal mixtures. The marked decreases of brain GPT were noticed in case of manganese and zinc mixture at the level of the three doses while least affecting mixture was zinc and copper. However fish subjected to the high dose of iron and copper mixture died after few hours of subjection.

Table (33): Mean values of glutamate pyruvate transaminase (GPT) (IU/g tissue/min) activity of the brain of Oreochromis niloticus after exposure to three levels of mixtures of iron and manganese, iron and zinc, iron and copper, manganese and zinc, manganese and copper and zinc and copper for 7 days (7 animals were used per test).

	Control	Fe and Mn	Fe and Zn	Fe and Cu	Mn and Zn	Mn and Cu	Zn and Cu
Low dose							
Mean ± S.E.	0.985±0.05	0.590±0.03	0.653±0.02	0.720±0.02	0.390±0.002	0.740±0.03	0.743±0.03
Range	0.808—1.162	0.471—0.686	0.571—0.700	0.619—0.803	0.380—0.400	0.619—0.900	0.631—0.864
Significance		P<0.01	P<0.01	P<0.01	P<0.001	P<0.02	P<0.02
% of change		-40%	-33%	-26.9%	-60%	-24.8%	-24.5%
Intermediate dose							
Mean ± S.E.		0.440±0.01	0.449±0.02	0.305±0.01	0.195±0.001	0.446±0.03	0.489±0.03
Range		0.400—0.515	0.404—0.526	0.265—0.366	0.194—0.200	0.357—0.545	0.329—0.471
Significance		P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
% of change		-55%	-54%	-69%	-80%	-54.7%	-48.9%
High dose							
Mean ± S.E.		0.200±0.01	0.216±0.001	All fish	0.097±0.0006	0.184±0.003	0.238±0.02
Range		0.181—0.283	0.210—0.222	expired	0.095—0.100	0.173—0.200	0.183—0.294
Significance		P<0.001	P<0.001		P<0.001	P<0.001	P<0.001
% of change		-79.6%	-78%		-90%	-81%	-75.8%

Acid phosphatase (AP) activity:

The data presented in table (34) depict the mean level of acid phosphatase (AP) activity (kind and king units/g tissue) of the brain of O. niloticus after exposing the fish to three doses of mixtures of iron and manganese, iron and zinc, iron and copper, manganese and zinc, manganese and copper and zinc and copper for 7days as compared with that of the control group. It is clear that acid phosphatase activity of the brain of O. niloticus of the control group was 0.695±0.06 kind and king units/g tissue while that of those fish subjected to the low dose of the mixtures of iron and manganese, iron and zinc, iron and copper, manganese and zinc, manganese and copper and zinc and copper was 0.470±0.003, 0.495±0.008, 0.446±0.01, 0.479±0.005, 0.496±0.01 and 0.533±0.01 kind and king units/g tissue respectively. The data declare that there was a significant decrease of brain AP activity of all of the above mentioned mixtures of metals treated fish groups. The decrease was 32%, 28.7%, 35.8%, 31%, 28.6% and 23% respectively.

Similarly the intermediate dose of metal mixtures applied to the fish resulted in a more marked decrease of brain AP activity as compared with that of the control group. These levels reached 0.338±0.01, 0.323± 0.005, 0.332±0.006, 0.322±0.005, 0.388±0.01 and 0.293±0.02 kind and king units/g tissue respectively. These decreases were statistically significant ($P<0.01$) for all of the abovementioned mixtures of metal salts treated fish groups. They revealed 51%, 53.5%, 52%, 53.6%, 44% and 57.8% of the mean control level respectively.

The mean level of brain AP activity of the control group and of those fishes subjected to the high dose of the metal mixtures of iron and manganese, iron and zinc, manganese and zinc, manganese and copper and zinc and copper were 0.695±0.06, 0.259±0.01, 0.160±0.009, 0.128± 0.007, 0.144±0.009 and 0.179±0.003 kind and king units/g tissue respectively. These data show highly significant ($P<0.001$) decreases that reached 62%, 76.9%, 81.5%, 79% and 74% of the mean control level.

As it can be seen from the table, the mean level of brain AP activity was inhibited markedly due to treatment with the metal combination mixtures and this inhibition was a dose dependent.

Table (34): Mean levels of acid phosphatase (AP) (kind and king Units/g tissue) activity of the brain of Oreochromis niloticus after exposure to three levels of mixtures of iron and manganese, iron and zinc, iron and copper, manganese and zinc, manganese and copper and zinc and copper for 7 days (7 animals were used per test).

	Control	Fe and Mn	Fe and Zn	Fe and Cu	Mn and Zn	Mn and Cu	Zn and Cu
Low dose							
Mean ± S.E.	0.695±0.06	0.470±0.003	0.495±0.008	0.446±0.01	0.479±0.005	0.496±0.01	0.533±0.01
Range	0.537—0.930	0.457—0.480	0.470—0.533	0.405—0.500	0.455—0.495	0.464—0.550	0.477—0.617
Significance		P<0.02	P<0.05	P<0.02	P<0.05	P<0.05	P<0.05
% of change		-32%	-28.7%	-35.8%	-31%	-28.6%	-23%
Intermediate dose							
Mean ± S.E.		0.338±0.01	0.323±0.005	0.332±0.006	0.322±0.005	0.388±0.01	0.293±0.02
Range		0.300—0.383	0.303—0.343	0.312—0.357	0.300—0.340	0.353—0.409	0.219—0.433
Significance		P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01
% of change		-51%	-53.5%	-52%	-53.6%	-44%	-57.8%
High dose							
Mean ± S.E.		0.259±0.01	0.160±0.009	All fish	0.128±0.007	0.144±0.009	0.179±0.003
Range		0.216—0.300	0.126—0.197	expired	0.110—0.161	0.108—0.165	0.165—0.190
Significance		P<0.002	P<0.001		P<0.001	P<0.001	P<0.001
% of change		-62%	-76.9%		-81.5%	-79%	-74%

Alkaline phosphatase (AlP) activity:

The obtained data shown in table (35) represent the mean alkaline phosphatase (AlP) (kind and king units/g tissue) activity of the brain of O. niloticus after subjection to three levels of mixtures of iron and manganese, iron and zinc, iron and copper, manganese and zinc, manganese and copper and zinc and copper for 7days together with that of the control group. The mean brain activity of AlP of the control group was 1.587±0.10 kind and king units/g tissue while those values of fishes exposed to the low concentration of the previously cited metal mixtures were 1.085±0.02, 0.913±0.01, 1.111±0.02, 0.844±0.01, 1.140± 0.03 and 1.330±0.02 kind and king units/g tissue. It is clear that there was a decrease of the mean brain AlP activity which was significant for all of the groups of fish except for those treated with zinc and copper mixture which was insignifiantly decreased. These decreases of the enzyme activity attained 31.6%, 42%, 29.9%, 46.8%, 28% and 16% respectively.

It is evident from the same table (Table 35) that brain AlP of O. nilotjcus treated with the intermediate dose of the paired metals was markedly decreased. This level was amounted to 1.587±0.10 for the control group yet, it was 0.965±0.01, 0795±0.02, 0.914±0.02, 0.634± 0.01, 0.893±0.02 and 0.778±0.04 kind and king units/g tissue for the mixtures of iron and manganese, iron and zinc, iron and copper, manganese and zinc, manganese and copper and zinc and copper treated groups respectively. These decreases of the enzyme activity were significant. The decreases amounted to 39%, 49.9%, 42%, 60%, 43.7% and 50.9% of the mean control value respectively.

The mean brain AlP activity of O. niloticus subjected to the high dose level of the combined metals (iron and manganese, iron and zinc, manganese and zinc, manganese, and copper and zinc and copper) was 0.415±0.01, 0.397±0.02, 0.358±0.01, 0.655±0.05 and 0.587±0.06 kind and king units/g tissue respectively. These data clearly show that the decrease was more deeper and statistically highly significant ($P<0.001$) for all of the forementioned mixtures. The decreases amounted to 73.8%, 74.9%, 77%, 58.7% and 63% of the mean control value respectively.

As it can be seen from table (35) that there was a more deeper decrease of brain AlP activity with increasing the dose of the paired metals and this decrease was a dose dependent. The high iron and copper mixture dose treated fish was a lethal one. It is clear also that manganese and copper mixture was of least effect and manganese and zinc mixture has the highest effect.

Table (35): Mean levels of alkaline phosphatase (AlP) (kind and king Units/g tissue) activity of the brain of Oreochromis niloticus after exposure to three levels of mixtures of iron and manganese, iron and zinc, iron and copper, manganese and zinc, manganese and copper and zinc and copper for 7 days (7 animals were used per test).

	Control	Fe and Mn	Fe and Zn	Fe and Cu	Mn and Zn	Mn and Cu	Zn and Cu
Low dose							
Mean ± S.E.	1.587±0.10	1.085±0.02	0.913±0.01	1.111±0.02	0.844±0.01	1.140±0.03	1.330±0.02
Range	1.157—1.935	1.028—1.176	0.873—0.980	1.000—1.160	0.792—0.882	1.000—1.300	1.235—1.444
Significance		P<0.01	P<0.01	P<0.01	P<0.02	P<0.02	insig.
% of change		-31.6%	-42%	-29.9%	-46.8%	-28%	-16%
Intermediate dose							
Mean ± S.E.		0.965±0.01	0.795±0.02	0.914±0.02	0.634±0.01	0.893±0.02	0.778±0.04
Range		0.918±1.030	0.721±0.851	0.825—0.990	0.588—0.679	0.803—0.982	0.571—0.909
Significance		P<0.01	P<0.002	P<0.01	P<0.001	P<0.01	P<0.002
% of change		-39%	-49.9%	-42%	-60%	-43.7%	-50.9%
High dose							
Mean ± S.E.		0.415±0.01	0.397±0.02	All fish	0.358±0.01	0.655±0.05	0.587±0.06
Range		0.363—0.471	0.315—0.444	expired	0.297—0.400	0.400—0.782	0.373—0.776
Significance		P<0.001	P<0.001		P<0.001	P<0.002	P<0.001
% of change		-73.8%	-74.9%		-77%	-58.7%	-63%

Effect of paired metals on some haematological aspects:

a) Erythrocyte count:

The data shown in table (36) present the mean erythrocyte count of the blood of Oreochromis niloticus after exposure to the low dose of iron and manganese, iron and zinc, iron and copper, manganese and zinc, manganese and copper and zinc and copper mixtures (as salts) for a one week period. The mean RBC was 3.80±0.43, 3.13±0.03, 3.60± 0.22,3.15±0.03, 3.16±0.11 and 3.06±0.03 $X10^6$cmm. respectively compared with 2.50±0.2 $X10^6$cmm for that of the control fish group. It is clear that there was a significant increase of the mean RBC count for all of the forementioned metal mixtures treated groups. These increases reached 52%, 25%, 44%, 26%, 26% and 22% respectively.

It is evident from the table also that the intermediate metal mixture doses applied to the fish groups caused highly significant increases of RBC counts (Table36) for all of the paired metals investigated. The increases reached 68%, 26%, 66%, 33%, 38% and 24% of the corresponding control value respectively.

Similarly the high metal mixture doses of the paired metals; iron and manganese, iron and zinc, manganese and zinc, manganese and copper and zinc and copper applied to the fish groups resulted in an increase of RBC count. The mean count was 4.31±0.14, 3.26±0.07, 4.10±0.03, 3.81+0.14 and 3.13±0.02 $X10^6$cmm respectively compared with 2.5±0.20 $X10^6$cmm for the control group. These increases were

statistically significant with a percentage of 72%, 30%, 64%, 52% and 25% respectively.

As it can be seen from the data, the three doses of the metal mixture groups applied to the fish resulted in a marked increase of erythrocyte count of the Nile fish; Oreochromis niloticus.

Table (36): Mean values of red blood cell count (RBC) ($x10^6$) of the blood of Oreochromis niloticus after exposure to three concentration levels of mixtures of iron and manganese, iron and zinc; iron and copper, manganese and zinc, manganese and copper and zinc and copper for 7 days(7 animals were used per test).

	Control	Fe and Mn	Fe and Zn	Fe and Cu	Mn and Zn	Mn and Cu	Zn and Cu
Low dose							
Mean ± S.E.	2.50±0.20	3.80±0.43	3.13±0.03	3.60±0.22	3.15±0.03	3.16±0.11	3.06±0.03
Range	2.10—3.20	3.1—5.2	3.0—3.255	3.0—4.3	3.1—3.3	3.0—3.75	3.0—3.2
Significance		P<0.005	P<0.05	P<0.02	P<0.05	P<0.05	P<0.05
% of change		+52%	+25%	+44%	+26%	+26%	+22%
Intermediate dose							
Mean ± S.E.		4.2±0.053	3.15±0.06	4.16±0.02	3.33±0.14	3.45±0.2	3.10±0.03
Range		4.0—4.4	3.0—3.45	4.1—4.17	3.0—4.0	3.0—4.3	3.0—3.2
Significance		P<0.002	P<0.05	P<0.002	P<0.05	P<0.05	P<0.05
% of change		+68%	+26%	+66%	+33%	+38%	+24%
High dose							
Mean ± S.E.		4.31±0.14	3.26±0.07	All fish	4.10±0.03	3.81±0.14	3.13±0.02
Range		4.1—5.1	3.0—3.5	expired	4.0—4.2	3.3—4.2	3.0—3.2
Significance		P<0.002	P<0.05		P<0.002	P<0.01	P<0.05
% of change		72%	+30%		+64%	+52%	+25%

b) Blood haemoglobin:

The data presented in table (37) depict the mean values of blood haemoglobin (Hb) (g/100 ml) of Oreochromis niloticus after treatment with the three concentration levels of mixtures of iron and manganses, iron and zinc, iron and copper, manganese and zinc, manganese and copper and zinc and copper for one week period. It is evident from the table that the mean value of Hb content of the control fish group was 8.51±0.13 g/100 ml whereas that for those fish raised in water containing the low dose of the of the paired metals was 11.83±0.28, 11.05±0.44, 12.0±0.23, 11.43±0.65, 9.66±0.19 and 11.16±0.43 g/100 ml respectively. Statistically, it is clear that the increase of mean Hb content of O. niloticus was significant for all the forementioned metal mixtures treated groups. These increases amounted 39%, 29.8%, 41%, 34%, 13.5% and 31% of the mean control value respectively.

With regards to the mean Hb content of the blood of O. niloticus treated with the intermediate mixture dose it was significantly increased when compared with that of the control group (Table 37). The mean Hb content reached 12.66±0.65, 11.26±0.46, 13.50±0.51, 12.0±0.26, 11.0± 0.33 and 12.50±0.20 g/100 ml respectively. The increase reached 48.7% in case of iron and manganese, 32% in case of iron and zinc, 58.6% in case of iron and copper, 41% in case of manganese and zinc, 29% in case of manganese and copper and 46.8% in case of zinc and copper mixture treated groups respectively.

The mean Hb content of O. niloticus was markedly increased also after subjecting the fish groups to the high dose of the paired metals, iron and manganese, iron and zinc, manganese and zinc, manganese and copper and zinc and copper, where the mean values reached 12.83±0.43, 13.63±0.28, 12.48±0.37, 12.50±0.31 and 12.83±0.34 g/100 ml respectively. These increases were statistically highly significant ($p < 0.001$) for all of the forementioned metal mixture treated groups respectively. Such increases reached 50.7%, 60%, 46.6%, 46.8% and 50.7% respectively.

It is clear from the previously cited data that applying any of the three doses of the four metal combinations resulted in a marked increase of blood Hb of O. niloticus. The highest increase was recorded for the intermediate dose mixture of iron and copper treated group and the least one was that of manganese and copper treated one.

Table (37): Mean values of haemoglobin level (Hb) (g/100 ml) of the blood of Oreochromis niloticus after exposure to three concentration levels of mixtures of iron and manganese, iron and zinc, iron and copper, manganese and zinc, manganese and copper and zinc and copper for 7 days (7 animals were used per test).

	Control	Fe and Mn	Fe and Zn	Fe and Cu	Mn and Zn	Mn and Cu	Zn and Cu
Low dose							
Mean ± S.E.	8.51±0.13	11.83±0.28	11.05±0.44	12.0±0.23	11.43±0.65	9.66±0.19	11.16±0.43
Range	8.0—8.9	11.0—13.0	9.8—12.0	11.0—13.0	9.0—13.6	9.0—10.0	10.0—13.0
Significance		P<0.001	P<0.001	P<0.001	P<0.002	P<0.01	P<0.01
% of change		+39%	+29.8%	+41%	+34%	+13.5	+31%
Intermediate dose							
Mean ± S.E.		12.66±0.65	11.26±0.46	13.50±0.51	12.0±0.26	11.0±0.33	12.50±0.20
Range		10.0—15.0	9.0—12.0	12.0—15.0	11.0—12.5	10.0—12.0	12.0—13.0
Significance		P<0.01	P<0.01	P<0.001	P<0.001	P<0.002	P<0.001
% of change		+48.7%	+32%	+58.6%	+41%	+29%	+46.8%
High dose							
Mean ± S.E.		12.83±0.43	13.63±0.28	All fish	12.48±0.37	12.50±0.31	12.83±0.34
Range		12.0—15.0	12.0—14.0	expired	11.0—13.3	12.0—14.0	12.0—14.5
Significance		P<0.001	P<0.001		P<0.001	P<0.001	P<0.001
% of change		+ 50.7%	+60%		+46.6%	+46.8%	+50.7%

Haematocrit value (HC%):

Table (38) shows the data obtained for packed cell volume of the blood cells of Oreochromis niloticus after treatment with the three doses of mixtures of iron and manganese, iron and zinc, iron and copper, manganese and zinc, manganese and copper and zinc and copper for a one week period. As it can be depicted from the table there was a marked increase in case of the low dose of the metal mixtures containing water. The mean HC value of the control fish was 23.0±0.50 which increased to reach 27.83±0.36, 28.0±0.33, 30.05±0.39, 25.50±0.20, 27.50±0.51, 29.66±0.19 for the above mentioned metal mixture groups respectively. Statistical analysis showed that the increase of the mean HC value was highly significant when compared with that of the control group. The percentage of increase was 21%, 21.7%, 30.6%, 10.8%, 19.5% and 28.9% respectively.

However, the intermediate metal mixture dose subjected fish groups revealed a deeper increase of HC values. The mean values were 30.66± 0.65, 30.0±0.52, 32.23+0.11, 27.0±0.40, 29.16±0.36 and 31.66±0.65% respectively. It is clear that these increases of the HC value were statistically highly significant ($p<0.001$) for all the metal mixture groups respectively. The percentage of increases were 33%, 30%, 40%, 17%, 26.7% and 37.6% respectively (Table 38).

HC value of O. niloticus was markedly increased after treating the fish groups with any of the high dose of metal mixtures. The mean values were 33.26±0.54, 32.66±0.50, 29.0±0.20, 29.83±0.49, 32.83±0.72% for

the metal combinations of iron and manganese, iron and zinc, manganese and zinc, manganese and copper and zinc and copper respectively. Statistically all of the obtained values were highly significanly ($p<0.001$) increased. The increases were 40%, 42%, 26%, 29.6% and 42.7% of the mean control value respectively.

It is clear that combination of the four metals with each other at the three different doses resulted in a significant increase of the mean HC value when compared with that of the control group.

Table (39): Mean corpuscluar volume (MCV), mean corpuscular haemog lobine (MCH) and mean corpuscular heamoglobin concentration (MCHC) of Oreochromis niloticus treated with paired metals (iron and manganese, iron and zinc, iron and copper, manganese and zinc, manganese and copper and zinc and copper) atthree dose levels for one week period.

	MCV			MCH			MCHC		
	low dose	intermediat dose	High dose	low dose	intermediat dose	High dose	low dose	intermediat dose	High dose
Control		92			33.04			37	
Feand Mn	73.2	63.0	77.2	31.1	30.1	29.8	42.5	41.3	38.6
Fea and Zn	89.5	95.2	77.2	35.3	35.7	41.8	39.5	37.5	41.7
Fe and Cu	83.5	77.5	-	33.3	32.5	-	39.9	41.9	-
Mn and Zn	80.9	81.1	70.7	36.3	36	30.4	44.8	44.4	43.0
Mn and Cu	87.6	84.5	76.3	30.6	31.9	32.8	35.1	37.7	41.9
Zn and Cu	96.9	102.1	104.9	36.5	30.3	40.99	37.6	39.5	39.1